Design, Synthesis and Biological Evaluation of Selective Nonpeptide AT2 Receptor Agonists and Antagonists

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Abstract

The G protein-coupled receptors (GPCRs) are important targets in drug discovery. In several cases, the endogenous ligands that activate the GPCRs of pharmaceutical interest are peptides. Unfortunately, peptides are in general not suitable as drugs, since the peptide structure is associated with several disadvantages, such as low oral bioavailability, rapid degradation and low receptor subtype selectivity. Thus, there is a strong need for drug-like nonpeptide ligands to peptide-activated GPCRs. However, to discover nonpeptide ligands that mimic the effect of the endogenous peptide, i.e. peptidomimetics, is a tremendous challenge. In fact, morphine and the related opioids were the only known examples of peptidomimetics before 1995 and these ligands were known long before the native endogenous peptide ligands were discovered.

The main objective of the work described in this thesis was to design, synthesize and biologically evaluate selective nonpeptide agonists to the peptide-activated GPCR AT2. The AT2 receptor belongs to the renin–angiotensin system, where the octapeptide angiotensin II (Ang II) is the major effector peptide. Ang II mediates its effects through the two GPCRs AT1 and AT2. The AT1 receptor is already an established target in the treatment of hypertension. The physiological role of the AT2 receptor, which is up-regulated in certain pathological conditions, is not fully understood but it seems to include positive effects such as vasodilatation, tissue repair, tissue regeneration and neuronal differentiation.

In the current investigation we started from the nonpeptide and nonselective (AT1/AT2) compound L-162,313. This ligand is a known AT1 receptor agonist but its effect on the AT2 receptor was unknown at the start of this project. We were able to show that it acts as an agonist also at the AT2 receptor. Furthermore, stepwise synthetic modifications of L-162,313 led to the identification of the first selective nonpeptide AT2 receptor agonist. Following the discovery of this compound several selective nonpeptide AT2 receptor agonists were identified. It was also revealed that a minor structural alteration of one of these compounds interconverted the functional activity from agonism to antagonism. The structural requirement for agonism vs antagonism was therefore studied. The functionality switch was suggested, at least partly, to be due to the spatial relationship between the methyleneimidazole group and the isobutyl side chain of the compounds. To further investigate the bioactive conformation(s) of this series of compounds enantiomerically pure analogues with conformationally constrained isobutyl chains were prepared. This study revealed that the direction of the isobutyl side chain determine whether the compounds act as agonists or antagonists at the AT2 receptor. Further investigations are required to fully elucidate the bioactive conformation(s) of these nonpeptide AT2 receptor agonists.

We believe that the selective nonpeptide AT2 receptor agonists and antagonists identified in this thesis will serve as important research tools in the continuing investigation of the physiological role of the AT2 receptor. We also believe that these drug-like compounds might provide potential leads in drug discovery processes.

Keywords: AT2 receptor agonist, AT2 receptor antagonist, Ang II peptidomimetics

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This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


Corrections, submitted.

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### Abbreviations

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<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
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<tr>
<td>Ang</td>
<td>Angiotensin</td>
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<td>Arg</td>
<td>Arginine</td>
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<tr>
<td>Asp</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome 450</td>
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<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIEA</td>
<td>$N,N$-diisopropylethylamine</td>
</tr>
<tr>
<td>DISCO</td>
<td>Distance comparison</td>
</tr>
<tr>
<td>DMAP</td>
<td>Dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HTS</td>
<td>High throughput screening</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibitor concentration giving 50% inhibition</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>$K_i$</td>
<td>Inhibition constant</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activate proteine kinase</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MW</td>
<td>Microwaves</td>
</tr>
<tr>
<td>$n$-BuLi</td>
<td>$n$-butyl lithium</td>
</tr>
<tr>
<td>NMP</td>
<td>$N$-methyl-2-pyrrolidone</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PEPPSI</td>
<td>Pyridine-enhanced precatalyst preparation stabilization and initiation</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
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<td>Phe</td>
<td>Phenylalanine</td>
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<td>PKG</td>
<td>cGMP-dependent protein kinases</td>
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<tr>
<td>Pro</td>
<td>Proline</td>
</tr>
<tr>
<td>RAS</td>
<td>Renin–angiotensin system</td>
</tr>
<tr>
<td>Sar</td>
<td>Sarcosine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure–activity relationship</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>sGC</td>
<td>Soluble guanylyl cyclase</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
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<tr>
<td>Val</td>
<td>Valine</td>
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1 Introduction

1.1 G Protein-Coupled Receptors

The G protein-coupled receptors (GPCRs) constitute one of the largest protein families in the human genome. These receptors control many major physiological processes and they are crucial in the regulation of cell function. All known GPCRs consist of seven α-helical transmembrane domains that are connected with extracellular and intracellular loops. GPCRs mediate signals across the cell membrane by interacting with a wide variety of extracellular ligands, such as biogenic amines, amino acids, lipids, peptides, photons and odorants. Classically, this results in coupling to intracellular heterotrimeric G proteins which, in turn, activate secondary-messenger pathways that generate the physiological response. The activation process of GPCRs is still under investigation, but there is evidence that these receptors exist in equilibrium of inactive and active conformations. Due to this equilibration, many GPCRs have a basal or constitutive activity even without the interaction of the native ligand. The equilibrium, and the subsequent functional response, can be shifted by interactions with different ligands acting as agonists, antagonists or inverse agonists. These ligands will differ in their ability to stabilize either the active or the inactive conformation(s) of the receptor. Agonists will bind to the active conformation(s) while antagonists, or inverse agonists, will stabilize the inactive conformation(s). This will result in a shift of the functional response of the receptor, where agonists increase the response. A full agonist fully activates the receptor and a partial agonist induces a submaximal activation. Inverse agonists decrease the response compared to the basal activity while antagonists freeze the response at the basal level. Thus, in the case of elevated basal activity, a discrimination appears in the functional response between a neutral antagonist and an inverse agonist, as shown in Figure 1.

1.2 GPCRs in Drug Discovery

The development of receptor selective ligands acting as agonists and antagonists allows selective regulation of GPCR activity and modulation of cellular responses associated with different diseases. In fact, GPCRs are the target
structures for more than half of all modern drugs, but still these receptors represent only a fraction of all GPCRs available. Due to the importance of this protein family, there is an ongoing search for new drugs that can regulate GPCRs with combined potent efficacy and high selectivity. Historically, the drug discovery process of GPCR ligands has relied on screening for certain biological activities. The identified hit has then been modified according to the biological response rather than by rational design based on the target structure or the native ligand structure, since neither the responsible receptor nor its endogenous ligand were known. Even though we now have more possibilities for design, screening methods remain the most important tool in the discovery of new chemical entities acting on GPCRs. Today high throughput screening (HTS) is used to rapidly evaluate a large number of compounds for certain biological activities. HTS provides new hits that were previously never associated with the selected biological target. After selection of hits and subsequent structure–activity relationship (SAR) investigations, lead compounds are identified that are further optimized to achieve molecules with desired pharmacodynamic and pharmacokinetic properties.

Unfortunately, structural-based design in the near future is unlikely to be successful for GPCRs due to the lack of three-dimensional (3D) structures. To date only two GPCRs have been successfully crystallized and their 3D structure solved namely the rhodopsin receptor and the \( \beta_2 \)-adrenergic receptor. The crystallization of GPCRs is difficult partly attributed to that these receptors are integrated in the cell membrane. Clues about the target structure of GPCRs have so far mainly relied on homology modeling and site-directed mutations. Another aspect that complicates the structural-based design of GPCR ligands is their multiple conformational states. To design an agonist the activated conformation should be used and for antagonist design

**Figure 1.** A hypothetical illustration of the functional response of a full agonist, a partial agonist, a neutral antagonist and an inverse agonist, in relation to the basal receptor activity.
the inactive conformation is needed to elucidate the important interactions for a ligand-stabilizing effect.\textsuperscript{5,6}

Ligand-based drug design, on the other hand, has been more important in GPCR drug discovery processes. Modifications of the native ligand can lead to the identification of new drugs selective for receptor subtypes and with either agonistic or antagonistic activity. One well-known example is the modifications of the native ligands to the adrenergic receptors, epinephrine and norepinephrine. SAR investigations of epinephrine and norepinephrine have resulted in ligands selective for the different adrenergic receptor subtypes with both agonistic and antagonistic activities. Figure 2 exemplifies modification of epinephrine that renders the $\beta_2$-selective agonist terbutaline and the $\beta_1$-selective antagonist metoprolol. However, for several hundred of the human GPCRs, the endogenous ligands are peptides or proteins.\textsuperscript{9} Unfortunately, the peptide structure of these native ligands is associated with several disadvantages in a drug perspective. Peptides exhibit low oral bioavailability, rapid degradation and low receptor subtype selectivity. There are only a few successful examples of peptides used as drugs e.g. insulin and oxytocin. Insulin, which is administered through injection is of tremendous therapeutic importance and is probably the most well-known example. Obviously, administration of insulin as a tablet is highly desirable from a patient perspective, but so far there is no insulin peptidomimetic available that would allow oral administration. Oxytocin cannot be administered orally either, but there is a formulation for nasal administration on the market, as well as injectables. The peptide-activated GPCRs are of great interest and the discovery of novel nonpeptide ligands with favourable pharmacokinetic properties to these receptors is a highly prioritized field in medicinal chemistry.\textsuperscript{9}

![Figure 2](image-url)

**Figure 2.** The nonselective endogenous adrenergic agonist epinephrine and the modified analogues terbutaline, which acts as a $\beta_2$-selective agonist, and metoprolol, which acts as a $\beta_1$-selective antagonist.
1.3 Nonpeptide Ligands to GPCRs

Several examples of nonpeptide ligands to peptide-activated GPCRs have already been developed. The majority of these ligands act as antagonists or inverse agonists. The development of nonpeptide ligands mimicking the effect of the endogenous peptide ligand and thereby acting as agonists has proven to be much more difficult. Compounds that mimic the effect of the peptide are referred to as peptidomimetics. One of the most well-known peptidomimetics is morphine, which is an agonist to the opioid receptors. Morphine was used to relieve pain long before the native peptide ligands, enkephalins, dynorphins and endomorphines were discovered. Until 1995, morphine and related opioids were the only examples of nonpeptide agonists acting on peptide-activated GPCRs, but more recently several examples have been disclosed. There are two main strategies applied in the search for peptidomimetics to peptide-activated GPCRs. The first takes advantage of the structure of the endogenous peptide ligand and the second is based on nonpeptide structures, mainly identified by screening methods. The two strategies will be briefly described in the following sections.

1.3.1 The Peptide Ligand Design Strategy

In the search for peptidomimetic ligands the endogenous peptide can be used as a starting point. The peptide ligand contains valuable information and clues for drug discovery even though it is not suitable as a drug itself. To extract the information, an iterative process is used where pharmacophoric groups in the peptide are identified by systematic evaluation of each amino acid residue through amino acid scans and truncations. The bioactive conformation of the highly flexible peptide is then probed by conformationally constrained pseudopeptides through cyclizations and the introduction of secondary structure mimetics. Finally, the key features of the endogenous peptide can be transferred to a rigid nonpeptidic scaffold that allows the proper spatial orientation of the pharmacophoric groups. The successful development of a somatostatin peptidomimetic using this strategy is illustrated in Figure 3, and this method will be further exemplified in section 2.3.2.
Figure 3. The endogenous peptide ligand somatostatin was modified into a potent cyclic hexapeptide somatostatin agonist that in turn could be modified to a nonpeptide somatostatin peptidomimetic.20

1.3.2 The Nonpeptide Ligand Design Strategy

The conversion of peptides to nonpeptide drug candidates is, however, very difficult, and the identification of peptidomimetics is not guaranteed.16 An alternative approach to the peptide ligand design strategy is the use of a nonpeptide starting structure. The nonpeptide starting structure can be derived from HTS or already existing ligands to the GPCR of interest. The identified ligand can then be modified by combinatorial chemistry, classical medicinal chemistry and frequently by applying diversity through computational methods including molecular modeling to achieve high affinity, selectivity, and the desired functionality.9,14,15 The nonpeptide ligand design strategy has proven to be more successful in the identification of nonpeptide antagonists to peptide-activated GPCRs, while the number of agonists still remain fairly low.9,15

In 1988, Evans et al. introduced the term ‘privileged structures’21, which has become an accepted concept in the discovery of nonpeptide drug candidates.4,19 A privileged structure is a structural fragment that is able to provide ligands with high affinity to more than one type of receptor.4,21 These fragments are frequent in nonpeptide ligands targeting GPCRs as well as other receptors and enzymes.19 Some examples of common privileged structures are diphenylmethane, biphenyl, benzodiazepine, 4-arylpirperazines, 4-arylpirperidines and spiro versions of the latter.19 Figure 4 shows some examples of nonpeptide antagonists comprising privileged structures. It has been
suggested that one beneficial effect of the privileged structures is their inherent resistance to hydrophobic collapse, which allows extensive hydrophobic interaction with the receptor that otherwise could be lost in intramolecular interactions. It has also been suggested that there is a corresponding privileged binding pocket in GPCRs, in which these fragments bind. Consequently, the privileged structures can act as an anchoring core structure and the decoration of this core can induce selectivity between receptors.

1.3.3 Interconversion of functionality

In a few examples of nonpeptide agonists to peptide-activated GPCRs, it has been shown that a minor structural alteration can result in an interconversion of the functional activity. The first example was again found in the opioids. Replacing the \( N \)-methyl substituent in morphine with an \( N \)-allyl, provides the potent opioid antagonist nalorphine, as can be seen in Figure 5. This kind of close structural relationship between nonpeptide agonists and antagonists to the same GPCR has later been disclosed in a few more cases, for example the CCK-A agonist shown in Figure 5 and the AT\(_1\) receptor agonist L-162,782 (Figure 13), which will be further discussed in section 2.4.4.

![Figure 4](image-url) Some examples of nonpeptide antagonists targeting GPCRs that comprise different privileged structures.
Figure 5. Top; the structures of the nonpeptide opioid agonist morphine, the structurally very similar antagonist nalorphine and one of the endogenous peptide ligands, Leu-enkephalin.\textsuperscript{14,18} Bottom; the nonpeptide CCK-A agonists, the structurally very similar antagonists and the C-terminal octapeptide of the endogenous peptide cholecystokinin (CCK), CCK-8, which is the minimum sequence required for agonism.\textsuperscript{23}
2 Angiotensin II

The endogenous peptide angiotensin II (Ang II, Asp$^1$-Arg$^2$-Val$^3$-Tyr$^4$-Ile$^5$-His$^6$-Pro$^7$-Phe$^8$) is the major effector peptide in the renin–angiotensin system (RAS). The RAS plays an important role in the regulation of blood pressure and fluid and electrolyte homeostasis. Ang II is formed through stepwise cleavage of the protein angiotensinogen. First renin cleaves off the inactive decapeptide angiotensin I (Ang I), and then Ang I is cleaved by angiotensin converting enzyme (ACE) to form the active octapeptide Ang II (Figure 6).

In the degradation of Ang II, several biologically active fragments are formed: Ang III (Ang (2-8)) acts as an agonist at Ang II binding sites while Ang IV (Ang (3-8)) and Ang (1-7) act on other binding sites.$^{25}$ Ang II mediates its effects through stimulation of two receptor subtypes in the RAS, the AT$_1$ and the AT$_2$ receptors. Both these receptors belong to the GPCR family.$^3$ A schematic picture of the RAS and the effects mediated by its receptors is shown in Figure 6. The RAS has an important role in maintaining normal cardiovascular homeostasis, but an over-activation in the RAS can result in the development of hypertension and various cardiovascular diseases, such as congestive heart failure, coronary ischemia and renal insufficiency.$^{26}$ The importance of RAS as a target for handling hypertension became clear after in vivo evaluations of the peptidic Ang II antagonist saralasin (section 2.3.1). Saralasin was injected in hypertensive patients and induced a reduction in blood pressure (for a historical perspective see ref. $^{27}$).

In this way, the inhibition of Ang II was identified as a target for treating hypertension and this initiated the development of the ACE inhibitors. The first ACE inhibitor, captopril, was discovered through elegant design by Cushman and Ondetti and reached the market in 1982.$^{28}$ Unfortunately, ACE has more physiological functions than Ang II formation. It is also important for the degradation of, e.g. bradykinin and this lack of selectivity results in unwanted side effects, mainly dry cough.$^{29-31}$ A more selective way of inhibiting the effect of Ang II is to block the AT$_1$ receptor (Figure 6). The non-peptide AT$_1$ receptor antagonists were developed with the aim of retaining the good antihypertensive effects of the ACE inhibitors but avoiding the side effects.$^{30,31}$ Both the ACE inhibitors and the AT$_1$ receptor antagonists are today well-established drugs in the treatment of hypertension. There have also been several attempts to inhibit the enzyme renin, but only one compound, aliskiren, has reached the market.$^{32,33}$
2.1 The AT₁ Receptor

The AT₁ receptor was identified in 1970 and is a member of the rhodopsin-like GPCRs class A. In humans, the AT₁ receptor is mainly expressed in the vasculature, kidneys, heart, adrenals, and brain. The classical Ang II effects, such as vasoconstriction, retention of salt and water, release of aldosterone and vasopressin, cellular growth and proliferation and sympathetic activation are mediated through the AT₁ receptor. All these effects serve to raise the blood pressure, and the negative effects of an over-activation of RAS mentioned above, are due to an over-stimulation of the AT₁ receptor. Several nonpeptide AT₁ antagonists are used clinically for treatment of hypertension today. These will be further discussed in section 2.4.1.

2.2 The AT₂ Receptor

The existence of a receptor heterogeneity in the Ang II binding was suggested after the discovery that the Ang II binding in certain tissues was sensitive to the disulfide-reducing agent dithiothreitol (DTT), while the binding in other tissues was unaffected. The Ang II receptor heterogeneity was verified during the development of the first nonpeptide Ang II antagonists. Apart from the nonpeptide Ang II antagonists, the AT₂ selective synthetic peptidic ligands [4-NH₂-Phe⁶]Ang II and CGP-42112A (Figure 7) served also as important tools in the identification of the two receptor subtypes.
At this stage their functional activity at the AT₂ receptor was not known, CGP-42112A was later proven to be an agonist by Buisson et al. and Brechler et al. through studies of T-type calcium currents in NG108-15 cells and cellular cGMP levels in PC12W cells respectively. The cloning of the AT₂ receptor reported in 1993 was the final evidence of the existence of two distinct Ang II receptor subtypes.

Analogous to the AT₁ receptor, the AT₂ receptor is classified as a rhodopsin-like GPCRs class A receptor. Although Ang II is the endogenous ligand to both receptors, they share only 32-34% sequence identity. The AT₁ and the AT₂ receptors have different signalling pathways and different physiological effects. The physiological role of the AT₂ receptor is not yet fully understood, but there is a great interest in the function of this receptor. Many of the effects mediated by the AT₂ receptor oppose the effects mediated by the AT₁ receptor, e.g. vasodilatation and anti-proliferation. Other effects mediated by the AT₂ receptor include apoptosis and carbonate secretion from the duodenum. Stimulation of AT₂ receptors in neuronal cells leads to neurite outgrowth, which is a characteristic for neuronal differentiation. Furthermore, the AT₂ receptor is widely expressed in the fetal tissues, but the expression drops dramatically after birth, and it is therefore suggested that the AT₂ receptor is important in fetal development. In the healthy adult, the expression of the AT₂ receptor is low and mainly focused to specific tissues such as the adrenal glands, uterus, ovary, vascular endothelium, heart and distinct brain areas. Notably, in the adult, the AT₂ receptor is up-regulated during certain pathological conditions such as myocardial infarction, vascular injury, brain ischemia, renal failure and cutaneous wounds, and it has been postulated to have a tissue protective role and induce tissue repair.

The lack of easily available functional assays for the AT₂ receptor has limited the number of ligands with identified functional properties. The physiological and pharmacological evaluations of the AT₂ receptor have
mainly relied on the peptidic agonist CGP-42112A (Figure 7) and the non-peptide agonist PD 123,319 \(^\text{56}\) (section 2.4.2). Selective, drug-like AT\(_2\) receptor agonists are highly desirable tools for both a full understanding of the physiological role of the AT\(_2\) receptor and its potential as a drug target. Regarding its role in pathopharmacology there are, so far, some indications of beneficial effects on heart function after myocardial infarction through selective stimulation of the AT\(_2\) receptor.\(^\text{35}\) Furthermore, some of the positive effects of the selective AT\(_1\) receptor antagonists, such as vasodilatation and normalization of cardiovascular tissues, have been suggested to arise from Ang II stimulation of the unblocked AT\(_2\) receptor.\(^\text{57,58}\) Clinical studies of antihypertensive drugs have shown that therapies that allow a stimulation of the AT\(_2\) receptor reduce the risk of stroke, e.g. AT\(_1\) receptor antagonists.\(^\text{59,60}\) These effects could be further evaluated with selective nonpeptide AT\(_2\) receptor agonists.

2.3 Structure–Activity Relationship of Ang II

2.3.1 Ang II SAR at the AT\(_1\) Receptor

The SAR of peptide Ang II-analogues to the AT\(_1\) receptor has been thoroughly investigated through amino acid scans, truncations and cyclizations (for reviews see references \(^\text{61,62}\)). The different roles of the amino acid residues in Ang II when activating the AT\(_1\) receptor, are briefly summarized here and in Figure 8.\(^\text{63}\) The pharmacophoric groups for agonism at the AT\(_1\) receptor include Tyr\(^4\), His\(^6\), and Phe\(^8\), as well as the C-terminal carboxylate. These residues are also required for good binding, in addition to

![Figure 8. A brief overview of the SAR of Ang II at the AT\(_1\) receptor, modified from Hodges et al.\(^\text{63}\)](image-url)
the positively charged Arg. The amino acid residues in Val, Ile and Pro are important for conformational stability. Exchanging Phe for aliphatic amino acids provide AT receptor antagonists, the most studied are [Sar, Ile]Ang II and [Sar, Val, Ile]Ang II (saralasin), which provided important research tools in the early investigations of RAS.

There is no consensus regarding the bioactive conformation of Ang II when it binds to the AT receptor. Several cyclized analogues of Ang II, rendering a turn structure around Tyr, have been synthesized and proven to act as agonists at the AT receptor. These findings suggest the existence of a turn conformation around Tyr, but an extended receptor-bound conformation has also been suggested based on homology modeling and photoaffinity labeling. Ang II is suggested to mainly bind in the transmembrane region of the AT receptor.

2.3.2 Ang II SAR at the AT Receptor

The SAR of Ang II and its analogues in the binding to the AT receptor has not been as thoroughly studied as for the AT receptor. The investigations performed have also focused on receptor binding due to the lack of functional assays. Modifications of Ang II are surprisingly well tolerated in the binding to the AT receptor. Amino acid scans of Ang II have shown that no single amino acid is essential for receptor binding. The C-terminal pentapeptides (Ac-Tyr-Ile-His-Pro-Ile/Ile) retain agonistic function and the substitution of Phe for Ile enhances AT receptor affinity (Figure 9). This suggests that the C-terminal amino acids are important for binding and function.

The bioactive conformation of Ang II, when binding to the AT receptor, has been investigated through cyclizations, incorporation of secondary structure mimetics, and ligand modeling. Both γ- and β-turn mimetics have been incorporated in the Tyr region in Ang II and both approaches afforded ligands with good affinity and retained function, suggesting the existence of a turn structure. The majority of these pseudopeptides have been selective for the AT receptor. The incorporation of secondary structure mimetics has also allowed N-terminal truncations that afforded potent agonistic ligands, which further support the importance of the C-terminal for AT receptor affinity and agonism. Georgsson et al. have shown that pseudopeptide B, presumably mimicking the His-Pro-Ile, binds selectively to the AT receptor (Figure 9). The functional activity of B at the AT receptor has not been evaluated.

Homology modeling and site-directed mutagenesis studies have suggested an extended conformation of Ang II in the binding to the AT receptor. The existence of a turn structure and an extended conformation does not need to contradict each other. As for the AT receptor, Ang II is suggested to mainly bind to the transmembrane region of the AT receptor.
2.4 Nonpeptide Ligands to the AT₁ and AT₂ receptors

2.4.1 Nonpeptide AT₁ Receptor Antagonists

The development of nonpeptide AT₁ receptor antagonists originates from the polysubstituted imidazolylacetic acid derivatives, e.g. S-8307 (Figure 10), disclosed by Furukawa and co-workers at Takeda Chemical Industries. The Takeda compounds possessed a weak inhibitory effect on Ang II-induced vasoconstriction and they constitute the first examples of nonpeptidic compounds that interacted with the AT₁ receptor. These nonpeptide compounds were used as leads in the development of the first orally active nonpeptide Ang II receptor antagonist, losartan. Losartan reached the market in 1994 and shortly afterwards several similar nonpeptide AT₁ antagonists were developed, forming a new class of drugs called the “sartans” (see Figure 10 for some examples). As can be seen from the structures in Figure 10, most sartans contain the privileged structure biphenyl. It is suggested that the binding site of the sartans at the AT₁ receptor partly overlaps with the peptide binding site of the C-terminal in Ang II. This is, in fact, consistent with the ideas behind the design of both losartan and eprosartan. Today, the sartans are clinically important drugs in the treatment of hypertension.

2.4.2 Nonpeptide AT₂ Receptor Antagonists

The first series of nonpeptide compounds selective for the AT₂ receptor were substituted tetrahydroimidazopyridines, including PD 123,319 (Figure 11). The tetrahydroimidazopyridines were independently developed by Parke-Davis at the same time as losartan, with the aim of inhibiting the hypertensive effect of Ang II. The compounds turned out to be very poor...
inhibitors of Ang II-induced vasoconstriction, which afterwards could be explained by their selectivity for the AT$_2$ receptor. In 1993 Brechler et al. proved that PD 123,319 acts as an antagonist at the AT$_2$ receptor as deduced from studies of cellular cGMP levels.\textsuperscript{44} Due to the lack of antihypertensive activity, these compounds never reached the clinic, but they have been and still are important tools and reference compounds in the studies of the AT$_2$ receptor.\textsuperscript{96}

Based on the structure of the PD compounds, VanAtten et al. developed a series of substituted tetrahydroisoquinoline derivatives, including EXP801, with high selectivity for the AT$_2$ receptor.\textsuperscript{97} The diacylpiperazine L-159,686 is another example of a highly selective AT$_2$ receptor binding inhibitor (Figure 11).\textsuperscript{98}

\textbf{Figure 11.} The selective nonpeptide AT$_2$ receptor antagonists PD 123,319, EXP801 and L-159,686.
2.4.3 Balanced Nonpeptide AT$_1$/AT$_2$ Receptor Antagonists

After the discovery of the AT$_2$ receptor, there was a big interest in developing “balanced” antagonists with equal binding affinities to both the AT$_1$ and the AT$_2$ receptors. The idea was that a blockade of both receptors would reduce the risk of unwanted side effects caused by an increased Ang II stimulation of the AT$_2$ receptor. The risk of increased Ang II stimulation was hypothesized to be a result of an interruption of the negative feedback control of Ang II formation when blocking the AT$_1$ receptor.\textsuperscript{99,100} Even though the physiological role of the new receptor was not known, at that time this effect was seen as a potential risk. A blockade of both receptors would also more closely mimic the effect of the ACE inhibitors.\textsuperscript{30,31} The balanced antagonists disclosed are verified antagonists at the AT$_1$ receptor, while notably the function at the AT$_2$ receptor is unexplored or at least not disclosed, to the best of my knowledge. At the time, they were anticipated to act as antagonists at the AT$_2$ receptor as well, and functional assays for the AT$_2$ receptor were poorly developed.

The first dual Ang II antagonists were benzimidazoles, for example BIBS-39 (Figure 12). These compounds showed modest affinity and a 20-fold selectivity for the AT$_1$ receptor.\textsuperscript{30,100,101} At the same time, Merck developed the first balanced AT$_1$/AT$_2$ antagonist, L-159,689, which showed low nanomolar affinity for both receptors (Figure 12).\textsuperscript{100,102} These lead compounds were followed by many more, most of which were based on the biphenyl scaffold of the sartans. It was later shown that sulfonamides or sulfonylecarbamates generally increased the AT$_2$ affinity more than the tetrazole.\textsuperscript{100,103} The substituted heteroaromatic group was thoroughly investigated and four major compound classes were formed: benzimidazoles, imidazopyridines, quinazolinones and triazolinones (for some examples see Figure 12).\textsuperscript{100} From the balanced nonpeptide AT$_1$/AT$_2$ receptor antagonists several AT$_2$ selective binding inhibitors were disclosed.\textsuperscript{104,105} The substituted quinazolinone L-161,638 is one of the most potent and selective AT$_2$ receptor binding inhibitor originating from the balanced AT$_1$/AT$_2$ ligands (Figure 12).\textsuperscript{105} None of the balanced antagonists reached the market.
Figure 12. Some examples of balanced AT$_1$/AT$_2$ antagonists, and the structurally related AT$_2$ selective ligand L-161,638.

2.4.4 Nonpeptide AT$_1$ Receptor Agonists

In the development of the balanced AT$_1$/AT$_2$ receptor antagonists it was found that an alkyl substituent in the 4-position of the lower phenyl increased AT$_2$ receptor affinity.$^{106,107}$ This finding led to, by pure serendipity, the identification of the first nonpeptide AT$_1$ receptor agonist, L-162,313 (Figure 13).$^{24,108,109}$ The agonistic properties were retained when the thienyl-phenyl scaffold was changed to the biphenyl scaffold, as in compound L-162,782.$^{24}$ The agonistic function was very sensitive to structural alterations since the n-propyl analogue of L-162,782, compound L-162,389 exerted antagonism (Figure 13).$^{107}$ These two compounds only differ in one single methyl group, but they possess different functionalities in vivo.$^{24}$ The agonistic compounds L-162,313 and L-162,782 constituted the second examples of nonpeptide agonists to a peptide-activated GPCR. The only previous example was morphine and related opioids in the case of the opioid receptors, as mentioned previously in section 1.3.$^{14}$

After the discovery of L-162,313 and L-162,782, the first selective AT$_1$ receptor agonist, L-163,491, was discovered. L-163,491 is the meta-methoxybenzyl derivative of L-162,782 and exhibited an affinity of 1.4 nM to the AT$_1$ receptor and 101 nM to the AT$_2$ receptor with retained agonistic
activity at the AT₁ receptor. An enlargement of the isobutyl substituent therefore seems to disfavour an interaction with the AT₂ receptor.

Figure 13. Structures of the nonpeptide AT₁ receptor agonists L-162,313, L-162,782 and L-163,491 and the structurally similar AT₁ receptor antagonist L-162,389.

When the present study began it was not known whether the nonselective AT₁/AT₂ ligand L-162,313 that stimulates the AT₁ receptor, acted as an AT₂ receptor agonist or antagonist.
3 Aims of the Present Study

The work described in this thesis is part of an ongoing project with the objective to design, synthesize and biologically evaluate selective nonpeptide AT₂ receptor agonists.

The specific objectives of this study were:

- To determine the functional activity at the AT₂ receptor of the nonselective (AT₁/AT₂) nonpeptide AT₁ receptor agonist L-162,313.

- To design and synthesize AT₂ receptor selective agonists from the nonselective (AT₁/AT₂) nonpeptide AT₁ receptor agonist L-162,313.

- To investigate the SAR of nonpeptide AT₂ receptor ligands derived from L-162,313.

- To determine agonistic and antagonistic functions of selective nonpeptide AT₂ receptor ligands at the AT₂ receptor, using a neurite outgrowth assay in NG108-15 cells.

- To investigate the structural requirements for agonistic vs antagonistic functions in selective nonpeptide AT₂ receptor ligands.

- To postulate the bioactive conformation(s) of selective nonpeptide AT₂ receptor agonists.
Methods for Biological Evaluation of Nonpeptide AT_2 Receptor Ligands

The biological evaluation of nonpeptide AT_2 receptor ligands synthesized have included both affinity and functionality assays. The different methods used are briefly described below.

4.1 Binding Affinity Assays
The binding affinities at the AT_1 and the AT_2 receptor were determined using radioligand-binding assays measuring the displacement of [^{125}I]Ang II. For the displacement from AT_1 receptors, rat liver membranes were used and for the displacement from AT_2 receptors pig uterus myometrium were used. The endogenous ligand Ang II, the selective nonpeptide AT_1 antagonist losartan and the selective nonpeptide AT_2 antagonist PD 123,319 were used as reference compounds. All experiments were performed in triplicate and the results are presented as \( K_i \pm \text{SEM} \), if not otherwise stated.

4.2 Functional Assays
With the aim of developing selective nonpeptide AT_2 receptor agonists it has been crucial to determine the functional activity at the AT_2 receptor. This evaluation has been performed on selected compounds, due to the fact that the functional assays available for the AT_2 receptor are very resource and time consuming. We have mainly used an in vitro assay, neurite outgrowth, for the determination of agonism and antagonism and this method is described below. We have also used an in vivo assay, carbonate secretion, which is also briefly described below.

4.2.1 Neurite Outgrowth Assay in NG108-15 Cells
In the in vitro evaluation of the functionality of our selective nonpeptide AT_2 receptor ligands we have used the hybrid mouse neuroblastoma × rat glioma NG108-15 cell line. NG108-15 cells have served as a model system for the description of transmitter and hormone actions on neuronal tissues.
differentiated NG108-15 cells have a rounded shape and divide actively. In this state these cells express only the AT2 receptor and a three-day treatment with Ang II or the CGP-42112A, the AT2 selective peptidic agonist presented in section 2.2, induce neurite outgrowth, which is one of the steps in neuronal differentiation. The signalling pathways leading to neurite outgrowth involve a sustained increase in Rap1/B-Raf/p42/p44 \textsuperscript{mapk} activity and activation of the nitric oxide/guanylyl cyclase/cGMP pathway.\textsuperscript{115-117} (For a review on the role of the AT2 receptor in neuronal differentiation see ref. \textsuperscript{52}.) In our evaluations we have focused on the neurite outgrowth assay, since it shows the end point of the AT2 receptor stimulation. In this assay, subcultures were performed at subconfluency and the cells were treated once daily over three days. On the fourth day, the cells were examined under a phase contrast microscope and pictures were taken, enabling studies of the cell morphology. Figure 14 shows pictures of untreated control cells and Ang II-stimulated cells. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth and the result is reported as the percentage positive cells of the total number of cells in the picture. Each condition was tested in duplicate in three independent experiments and the results are presented as the mean value ± SEM. The effect of the tested compounds was compared to untreated cells and cells treated with Ang II, as negative and positive controls. Coincubation with the selective nonpeptidic AT2 receptor antagonist PD 123,319 was used to verify an agonistic effect at the AT2 receptor. In the same manner coincubation with Ang II was used to verify an antagonistic effect at the AT2 receptor.

Figure 14. (A) Example of the morphology of untreated control cells. (B) Example of the morphology of Ang II stimulated cells.

4.2.2 Carbonate Secretion Assay

Ang II induces duodenal mucosal alkaline secretion via stimulation of the AT2 receptor. The secretory response is blocked by the selective AT2 receptor antagonist PD 123,319, but it is unaffected by the selective AT1 receptor
antagonist losartan.\textsuperscript{50,118} The AT\textsubscript{2} receptor-mediated carbonate secretion in the duodenum can thus be used as an in vivo model for measuring AT\textsubscript{2} receptor agonism. The experiment is performed with anaesthetized rats and uses a pH-stat titration technique to measure the secretory response.\textsuperscript{119} The alkaline secretion is continuously titrated to pH 7.4 with 0.02 M aqueous hydrochloric acid, and the secretory response can thereby be determined by the amount of acid needed to maintain the pH at 7.4.\textsuperscript{119} The effect of different drugs on the carbonate secretion can be studied by i.v. administration or by adding the drug to the buffer (intraluminal administration) and use the pH-stat titration to measure the effect of the drug on the alkaline secretion.
5 Investigation of the Nonselective AT\textsubscript{1} Receptor Agonist L-162,313 (Paper I)

5.1 Background

With the aim of developing selective nonpeptide AT\textsubscript{2} receptor agonists, the nonselective AT\textsubscript{1} receptor agonist L-162,313 was selected as starting structure. L-162,313 is a nonpeptidic structure that shows similar affinities to both the AT\textsubscript{1} and the AT\textsubscript{2} receptor (Figure 15). The compound has been proven to act as an agonist at the AT\textsubscript{1} receptor, while the function at the AT\textsubscript{2} receptor has been unknown.\textsuperscript{24,108,109} As a first step we wanted to determine whether L-162,313 functioned as an agonist or an antagonist at the AT\textsubscript{2} receptor. The carbonate secretion assay showed that L-162,313 acted as an agonist also at the AT\textsubscript{2} receptor. This evaluation is further discussed in section 5.3.2. From a synthetic point of view the easiest position for modifications of L-162,313 is the sulfonylcarbamate group. Therefore we decided to investigate the impact of some minor alterations and synthesized a series of compounds, here designated as series A (Figure 15). None of the modifications in series A rendered good affinity to the AT\textsubscript{2} receptor. From the results obtained with the compounds in series A, in combination with the results in previous investigations\textsuperscript{100,103}, it seemed like the \textit{n}-butyl sulfonylcarbamate was the best choice for good AT\textsubscript{2} receptor affinity. Removal of a methyl group of the isobutyl substituent rendered an antagonist and the larger groups in this position disfavoured AT\textsubscript{2} receptor affinity but favoured AT\textsubscript{1} receptor affinity, as discussed in section 2.4.4.\textsuperscript{24,110} Therefore we focused on modifications of the heteroaromatic upper part of L-162,313, to investigate whether modifications in this region of the structure could induce AT\textsubscript{2} receptor selectivity with retained agonistic activity.

Several selective nonpeptide AT\textsubscript{2} receptor antagonists have been reported and many of them contain substituted quinazolinones instead of the imidazopyridine in L-162,313 (see for example L-161,638 in Figure 12).\textsuperscript{102,104,105,120-122} Consequently, the substituted quinazolinones must have a structural feature that discriminates between the AT\textsubscript{1} and the AT\textsubscript{2} receptors. We were therefore interested to see the effect on the selectivity pattern and functional activity after keeping the lower part of the balanced AT\textsubscript{1}/AT\textsubscript{2} agonist L-162,313 and exchanging the imidazopyridine for substituted quinazolinones. The combination of substituted quinazolinones with L-
162,313 resulted in a series of compounds, series B, which were evaluated for affinity to both receptors (Figure 15). The biological function was evaluated in one of the compounds.

![Figure 15. The structure and biological activities of L-162,313 and the general structures for the synthesized compounds in series A and B.](image)

### 5.2 Synthesis

The synthesis of L-162,313, series A and series B all starts with the separate synthesis of two key intermediates, the substituted thiophene and the para substituted benzene. These intermediates are then coupled together by a carbon-carbon bond formation reaction, to form the thienylphenyl scaffold. Efficient methods that allow carbon-carbon bond forming reactions to occur smoothly are very important in organic synthesis and among the most straightforward and general reactions are the cross-couplings between organic electrophiles and organometallic compounds. One of the milder and more user-friendly cross-couplings is the Suzuki, or Suzuki-Miyaura cross-coupling. In the Suzuki reaction an organoboron compound, usually a boronic acid or ester, is reacted with an organic electrophile, with palladium as catalyst, and in the presence of a base. The base increases the reactivity of the organic group attached to boron by coordination to the boron atom, which is postulated to facilitate transmetalation. In order to use the Suzuki cross-coupling for the formation of the thienylphenyl scaffold, the substituted thiophene was transformed into the corresponding boronic acid 3. Compound 3 was prepared essentially as described by Kevin et al., and was used in the synthesis of L-162,313, series A and series B. Thus, as outlined in Scheme 1, the synthesis started with the conversion of the commercially available thiophene-2-sulfonyl chloride to the \(N\)-\textit{tert}-butylsulfonamide, 1, by reaction with \(\text{\textit{tert}}\)-butylamine. Subsequent alkylation of the 5-position via lithiation rendered compound 2, which then was object
for a selective 3-lithiation followed by boronation and acidic hydrolysis to deliver the boronic acid 3.

Scheme 1.

The other key intermediate in the synthesis of L-162,313 and series A, the \textit{para} substituted benzene, was the aryl bromide 3-(4-bromobenzyl)-2-ethyl-5,7-dimethyl-3\textit{H}-imidazo[4,5-\textit{b}]pyridine, 7. Compound 7 served as the organic electrophile in the Suzuki reaction with boronic acid 3, and was synthesized according to Senanayake \textit{et al.} As outlined in Scheme 2, the reaction started with the condensation of malonamidine with 2,4-pentadione to yield the 2-amino-4,6-dimethylnicotinamide 4. The following Hofmann rearrangement with iodo benzene diacetate under basic conditions afforded the urea 5. The desired imidazopyridine 6 was achieved by treatment of 5 with propanoic acid and propanoic anhydride. The Lewis acid MgCl$_2$ was used to activate the urea carbonyl for the nucleophilic attack of propanoic acid. Compound 7 was afforded by N-alkylation of 6 with 4-bromobenzyl bromide under basic conditions.

Scheme 2.

The two key intermediates, the crude boronic acid 3 and the aryl bromide 7, were subsequently subjected to the Suzuki reaction conditions, using Pd(PPh$_3$)$_4$ as palladium source and NaOH as base to render 8 in good yield.
Deprotection of the sulfonamide 8 with TFA and a few drops of anisole as electrophilic scavenger, provided the primary sulfonamide, which served as a good handle for decoration. The primary sulfonamide was acylated with alkyl chloroformates and acyl chlorides in pyridine, using 4-pyrrolidinopyridine as nucleophilic catalyst, to afford compounds L-162,313 and 9–14 (Scheme 3).

Scheme 3.

In the synthesis of the substituted quinazolinones in series B the aryl bromide 3-(4-bromobenzyl)-6-nitro-2-propyl-3H-quinazolin-4-one, 17, was used in the Suzuki coupling with boronic acid 3. Compound 17 was synthesized according to the procedure of Allen et al. and is shown in Scheme 4. 2-amino-5-nitrobenzonitrile was acylated with butanoic anhydride to form 15. Compound 15 was then hydrolyzed and cyclized upon treatment with NaOH, hydrogen peroxide and heat to form the quinazolinone 16. Finally, 17 was formed by deprotonation of 16 with ethoxide and subsequent N-alkylation with 4-bromobenzyl bromide.

Scheme 4.

The derivatives in series B differ in the substitution of the 6-position of the quinazolinone and the synthesis of the compounds is outlined in
Scheme 5. The first step was the Suzuki coupling of the crude boronic acid 3 with aryl bromide 17, which rendered 18 in 82% yield. The undecorated nitro compound 19 was also interesting for evaluation and it was achieved in good yield by deprotection of 18 with TFA, followed by acylation with n-butyl chloroformate, as described above.

To obtain compounds 24–26 and 36–42 the nitro group in 18 was reduced to the amine with ammonium formate and palladium on charcoal to provide the intermediate 20. The secondary amides 24–26 were synthesized by acylation of the amine in 20 with acetyl chloride, benzoyl chloride and thienoyl chloride respectively, followed by deprotection and subsequent acylation of the primary sulfonamide with n-butyl chloroformate, as previously described.

The tertiary amides 36–42 were prepared by a three-step procedure starting from the amine intermediate 20. First the secondary amines 27 and 28 were achieved by a reductive alkylation of the amine in compound 20 with acetaldehyde or benzaldehyde, using triacetoxyborohydride as reducing agent. Secondly 27 and 28 were acylated to render the tertiary amides 29–35. These compounds were then taken through the final step with the deprotection and subsequent acylation of the sulfonamide to yield the final compounds 36–42.

5.3 Biological Evaluation

5.3.1 Binding Affinity to the AT₁ and the AT₂ Receptors

All compounds including L-162,313 were evaluated for affinity to both the AT₁ and the AT₂ receptors in the radioligand-binding assays described in section 4.1, and the results are presented in Table 1 and Table 2. L-162,313 showed very similar affinities in our assays (AT₂: $K_i = 2.8$ nM, AT₁: $K_i = 3.9$ nM, Table 1) as compared to the IC₅₀ values presented by Kivlighn et al. (AT₂: IC₅₀ = 2.0 nM, AT₁: IC₅₀ = 1.1 nM, Figure 15).108 As can be seen from the results of series A (Table 1) modifications in the sulfonylcarbamate part of L-162,313 is not tolerated by the AT₂ receptor. The only modification that retained some affinity for the AT₂ receptor was the shortening of the side chain with removal of the oxygen. This n-butyl acylsulfonamide, 14, possessed an AT₂ receptor affinity of 400 nM. All the other modifications resulted in a complete loss of AT₂ receptor affinity. From these results it seemed like the imidazopyridines were very sensitive to any changes in the sulfonylcarbamate part regarding affinity to the AT₂ receptor. It was slightly surprising that the effect was this deleterious, since several of these side chains, and also very similar side chains, have been used previously in AT₂ active compounds.127,128 Mantlo et al. have e.g. shown that both the
Scheme 5.

$n$-butyl and the cyclopentyl side chains resulted in high affinity compounds, but in our series this side chain, in 12, gave complete loss of affinity.\textsuperscript{128} Mantlo’s compounds were also imidazopyridines, but they contained an amide in position 6 on the imidazopyridine and it is probably this amide that makes the compounds more tolerant to changes in the sulfonamide substituents.\textsuperscript{128} Analogous to our results both Rivero \textit{et al.} and Kevin \textit{et al.} found that the AT\textsubscript{2} receptor affinity was very sensitive to small changes in the alkyl substituent in the 4-position and the 5-position on the lower phenyl and thiophene ring, respectively.\textsuperscript{106,107} Both in series A and in these previously investigated series\textsuperscript{106,107,127,128} the impact of these modifications on AT\textsubscript{1} receptor affinity was very small in comparison to the impact on AT\textsubscript{2} receptor affinity.
Table 1. In vitro binding affinities of L-162,313 and the compounds in series A.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>$K_1$ (nM)</th>
<th>AT₁/AT₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AT₂</td>
<td>AT₁</td>
</tr>
<tr>
<td>L-162,313</td>
<td></td>
<td>2.8 ± 0.3</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>&gt;10,000</td>
<td>34.9 ± 4.6</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>&gt;10,000</td>
<td>55.2 ± 1.2</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>&gt;10,000</td>
<td>121 ± 2.2</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>&gt;10,000</td>
<td>62.0 ± 2.0</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>&gt;10,000</td>
<td>109 ± 5.4</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>400 ± 15.8</td>
<td>33.9 ± 2.4</td>
</tr>
</tbody>
</table>

All compounds in series A possess AT₁ affinity, even though 10- to 40-fold lower than L-162,313.

In series B, as discussed above, we decided to keep the lower part of L-162,313 and focus on modifications of the bicyclic heteroaromatic upper part. Our hypothesis was that the introduction of substituted quinazolinones would increase the affinity and selectivity for the AT₂ receptor, since these structures previously have been reported to discriminate between the two receptor subtypes. This modification did increase the AT₂ receptor selectivity, and several compounds in series B possessed good affinity and moderate selectivity for the AT₂ receptor, as can be seen in Table 2. But
Table 2. In vitro binding affinities of the compounds in series B.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>( K_1 ) (nM) (\begin{array}{c} \Delta T_2 \ \Delta T_1 \end{array})</th>
<th>( \Delta T_1 / \Delta T_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Nitro</td>
<td></td>
<td>134 ± 9.8</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>24</td>
<td>H</td>
<td></td>
<td>16.7 ± 0.1</td>
<td>728 ± 19.8</td>
</tr>
<tr>
<td>25</td>
<td>H</td>
<td></td>
<td>84.8 ± 2.2</td>
<td>343 ± 26.8</td>
</tr>
<tr>
<td>26</td>
<td>H</td>
<td></td>
<td>&gt;10,000</td>
<td>455 ± 19.8</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td>4.6 ± 0.5</td>
<td>231 ± 6.8</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td></td>
<td>33.4 ± 2.7</td>
<td>410 ± 9.5</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td>8.0 ± 0.4</td>
<td>693 ± 12.8</td>
</tr>
<tr>
<td>39</td>
<td></td>
<td></td>
<td>48.2 ± 1.9</td>
<td>339 ± 5.6</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td>45.4 ± 5.8</td>
<td>185 ± 3.7</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td>31.5 ± 1.8</td>
<td>248 ± 9.0</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td>&gt;10,000</td>
<td>1040 ± 25.4</td>
</tr>
</tbody>
</table>
once again it was obvious that the AT₂ receptor is very sensitive to small structural modifications and that the SAR is not very clear. For example the phenyl and the thienyl gave similar AT₂ receptor affinity in the tertiary ethyl amides 37 and 38, but when the ethyl was exchanged for hydrogen or benzyl the thienyl resulted in complete loss of affinity, 26 and 42, while the phenyl retained almost the same affinity, 25 and 41. This big difference is somewhat notable since benzene and thiophene often are seen as good bioisosteres to each other, which can be seen in L-162,782 and L-162,313 (Figure 13). The best AT₂ receptor affinity in series B, 4.6 nM, was achieved by 36, which also possessed a 50-fold selectivity for the AT₂ receptor.

5.3.2 Functional Activity at the AT₂ Receptor
L-162,313 was evaluated in the duodenal mucosal alkaline secretion assay, described in section 4.2.2, to determine the functionality at the AT₂ receptor. The duodenal mucosal alkaline secretion was moderately increased by L-162,313 alone, but was more pronounced in combination with losartan (Figure 16). These effects are in agreement with the effects seen from the peptide agonists Ang II and CGP-42112A and indicate that L-162,313 is an agonist at the AT₂ receptor. The mean arterial blood pressure was measured in parallel to the carbonate secretion to monitor the agonistic effect of L-162,313 at the AT₁ receptor. As can be seen in Figure 16 the administration of L-162,313 alone (bolus i.v. 0.3 mg/kg plus 30 μg/kg × h) did increase the mean arterial pressure, by approximately 10 mmHg. This effect could be inhibited by the AT₁ selective antagonist losartan (10 mg/kg, bolus i.v.), verifying that the effect was mediated by a stimulation of the AT₁ receptor. The agonistic properties of L-162,313 at the AT₂ receptor was further investigated by topical administration of the compound at the secreting epithelium in the duodenum, where only the AT₂ receptor is expressed. The alkaline secretion increased in a concentration-dependent manner and the effect was inhibited by co-administration of PD 123,319 (Figure 17). These in vivo results strongly indicate that L-162,313 acts as an agonist at the AT₂ receptor. L-162,313 has later been evaluated in the neurite outgrowth assay, where it also was found to act as an AT₂ receptor agonist (unpublished data).
Figure 16. Effects of intravenous administration of L-162,313 (bolus 0.3 mg/kg plus 30 μg/kg × h) and later addition of the AT1 receptor antagonist losartan (10 mg/kg, bolus i.v.) on duodenal mucosal alkaline secretion and mean arterial pressure in anaesthetized Sprague–Dawley rats (n = 5).

From series B 24 was selected for functional evaluation in vivo. Compound 24 was one of the most potent and selective AT2 receptor ligands and it showed the most favourable solubility properties. In contrast to L-162,313, 24 did not show any increase in alkaline secretion in the rat duodenum. The result from this assay suggests that 24 is not an AT2 receptor agonist, but it is difficult to interpret since there were problems with the solubility. Compound 24 has later been evaluated in the neurite outgrowth assay, where it was found to act as a weak agonist (unpublished data).

Figure 17. Effects of locally (intra duodenal) administered L-162,313 at consecutively increased concentrations in 30 minutes periods in the absence (n = 5) and in the presence (n = 5) of the selective AT2 receptor antagonist PD 123,319 (0.1 mM) in anaesthetized Sprague–Dawley rats. The values represent means of the last 15 periods of each concentration.
6 Discovery of the First Selective Nonpeptide AT₂ Receptor Agonist (Paper II)

6.1 Design

From our previous results discussed in section 5.3, we knew that the lower part of L-162,313 was important for high AT₂ receptor affinity, and that it was possible to induce AT₂ selectivity through modifications of the heteroaromatic upper part. But an extension of the quinazolinone substituent resulted in poor solubility and reduced drug-likeness. In our continuous investigation we were therefore eager to reduce the size of our compounds. We returned to L-162,313 and decided to make a step-wise reduction of the imidazopyridine part to see how much we could remove of the structure without losing all affinity (series C, Figure 18). With this approach we hoped to find a new, smaller starting point where we again could make decorations and possibly find AT₂ receptor selectivity and agonistic activity. We also wanted to investigate the impact of the position of the nitrogen-containing substituent. Therefore we synthesized the thienylbenzyl scaffold instead of the thienylphenyl scaffold and attached the substituent directly to the benzene ring. In this way the number of atoms between the thiophene and the nitrogen-containing substituent on the benzene ring would be the same, but the orientation of the substituent would be radically changed (series D, Figure 18). As nitrogen-containing substituents we chose nitrile and imidazole.

Figure 18. The structure and biological activities of L-162,313 and the general structures for the synthesized compounds in series C and D.
6.2 Synthesis

The synthesis of series C is outlined in Scheme 6, and the route was, in general, very similar to the synthesis of L-162,313, starting with a Suzuki coupling of the boronic acid 3 and the different para substituted bromobenzenes. The bromobenzenes, 43–49, were prepared by N-alkylation of the appropriate heterocycle with 4-bromobenzyl bromide, under basic conditions and isolated in moderate to excellent yields. The formed bromobenzenes were coupled with the boronic acid 3 in a Suzuki reaction with Pd(PPh 3 ) 4 as catalyst and NaOH as base to achieve the sulfonamides 50–54. The sulfonamides 55 and 56 were achieved after Suzuki coupling with 4-bromophenylacetonitrile (48) and 4-bromobenzonitrile (49) respectively. The reactions were heated conventionally and yielded the products in moderate to excellent yields. Compound 54 was also prepared by an alternative procedure, using microwave heating (150 °C, 5 min) and Na 2 CO 3 as base in the Suzuki reaction. 130,131 This procedure was very fast and yielded 54 in 75% compared to 63% in the thermal heating. The sulfonamides 50–56 were deprotected by TFA or the by the milder deprotecting reagent BCl 3,132 to render the primary sulfonamides. The final products 57–63 were afforded after a subsequent acylation of the primary sulfonamides with n-butyl chloroformate as previously described. However, compound 60 needed slightly different conditions for the acylation due to formation of an acylimidazolium salt with the nucleophilic catalyst 4-pyrrolidinopyridine. Using the two-phase system DCM/H 2 O with Na 2 CO 3 as base prevented this problem.

Scheme 6.
The two compounds 66 and 68 in series D were synthesized as outlined in Scheme 7. The synthesis started with a Suzuki coupling of boronic acid 3 with 4-bromobenzyl bromide in toluene and ethanol, using Pd(PPh$_3$)$_4$ as palladium source and Na$_2$CO$_3$ as base. The weaker base was used to avoid substitution of the benzylic bromide by hydroxide forming the corresponding benzyl alcohol. The Suzuki coupling was performed both with conventional (reflux, 3 h) and microwave heating (110 °C, 30 min) and both conditions afforded 64 in good yields (80% and 78% respectively). Compound 64 was then used in two different reactions to introduce the nitrogen-containing aryl substituents. The nitrile in 65 was introduced by a microwave-enhanced palladium catalyzed cross coupling, using Zn(CN)$_2$ and Pd(PPh$_3$)$_4$ as palladium source. The reaction was fast (180 °C, 2 min) and provided 65 in good yield. The imidazole in 67 was introduced using the copper catalyzed Ullman coupling under microwave heating (180 °C, 40 min). The reaction
afforded 67 in only 27% yield. Compounds 65 and 67 were then deprotected using the milder deprotection reagent BCl$_3^{132}$ and subsequently acylated with n-butyl chloroformate as previously described, to achieve the final products 66 and 68.

6.3 Biological Evaluation

6.3.1 Binding Affinity to the AT$_1$ and the AT$_2$ Receptors

Compounds 57–63, 66 and 68 were evaluated for affinity to the AT$_1$ and the AT$_2$ receptors, as described in section 4.1, and the $K_i$ values are presented in Table 3 and 4. As can be seen in Table 3, the affinity of 57 to the AT$_1$ receptor was completely lost. The alkyl substituents on the imidazopyridine must therefore be crucial for the interaction with the AT$_1$ receptor. The affinity to the AT$_2$ receptor, on the other hand, was not largely affected by this modification (7.1 nM), which therefore results in a strong selectivity for the AT$_2$ receptor. In 58 we had exchanged the imidazopyridine for a benzimidazole and kept the ethyl chain in the 2-position and, as for 57, this compound showed no affinity to the AT$_1$ receptor while the affinity to the AT$_2$ receptor was slightly increased to 3.2 nM. Notably, when the ethyl substituent was removed from the benzimidazole, as in 59, affinity to the AT$_1$ receptor was regained but only to 500 nM. Compound 59 showed equal affinity to the AT$_2$ receptor as 58. When the nitrogen-containing heteroaromatic head consisted of only a methyl-imidazole and an imidazole, 60 and 61, respectively, the AT$_1$ affinity was lost again, but the AT$_2$ affinity was increased by a 10-fold to 0.5 nM and 0.4 nM respectively. When the structure was further simplified from imidazole to nitrile, 62, once again AT$_1$ receptor affinity was regained to a $K_i$ of 100 nM, while the AT$_2$ receptor affinity was reduced to 70 nM. Unexpectedly, the nitrile compound 62 was thus a balanced AT$_1$/AT$_2$ receptor ligand. The arylnitrile compound 63 lost all affinity to both receptors.

The thienylbenzyl scaffold in series D, 66 and 68, resulted in inactive compounds, as can be seen in Table 4. This modification is thus deleterious for the interaction with both receptors, since the corresponding thienylphenyl compounds, 62 and 61 respectively, showed good affinity to the AT$_2$ receptor and 62 also possessed affinity to the AT$_1$ receptor.

From these modifications we have afforded several potent, highly selective, nonpeptide AT$_2$ receptor ligands. The compound showing the highest affinity to and selectivity for the AT$_2$ receptor was compound 61.
Table 3. In vitro binding affinities of the compounds in series C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>$K_i^a$ (nM)</th>
<th>AT₂</th>
<th>AT₁</th>
<th>AT₁/AT₂</th>
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<tr>
<td>57</td>
<td><img src="image1" alt="Image" /></td>
<td>7.1</td>
<td>&gt;10,000</td>
<td>&gt;1400</td>
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<tr>
<td>58</td>
<td><img src="image2" alt="Image" /></td>
<td>3.2</td>
<td>&gt;10,000</td>
<td>&gt;3125</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td><img src="image3" alt="Image" /></td>
<td>4.0</td>
<td>500</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td><img src="image4" alt="Image" /></td>
<td>0.5</td>
<td>&gt;10,000</td>
<td>&gt;20,000</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td><img src="image5" alt="Image" /></td>
<td>0.4</td>
<td>&gt;10,000</td>
<td>&gt;25,000</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td><img src="image6" alt="Image" /></td>
<td>70</td>
<td>100</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td><img src="image7" alt="Image" /></td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*a Standard deviations are less than 15% in all cases.
Table 4. In vitro binding affinities of the compounds in series D.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₂</th>
<th>(K_i^a) (nM)</th>
<th>(\text{AT}_2)</th>
<th>(\text{AT}_1)</th>
<th>(\text{AT}_1/\text{AT}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>CN</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>68</td>
<td>N</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviations are less than 15% in all cases.

6.3.2 Functional Activity at the AT₂ Receptor
The most potent compound, 61, was selected for evaluation in terms of agonistic and antagonistic activity at the AT₂ receptor. The functional activity of 61 at the AT₂ receptor was evaluated in both in vitro and in vivo studies.

6.3.2.1 Neurite Outgrowth Assay in NG108-15 Cells
Compound 61 was evaluated in the neurite outgrowth assay on NG108-15 cells, as described in section 4.2.1. Three days stimulation with 61 induced the same morphological changes as Ang II. Coincubation of 61 with the AT₂ selective antagonist PD 123,319 reduced neurite outgrowth to control levels, verifying that the neurite outgrowth was mediated by stimulation of the AT₂ receptor. The results can be seen in Figure 19 A, and examples of the cell morphology in the different conditions are shown in Figure 20. To further investigate whether 61 induced the same signaling cascade as Ang II, 61 was coincubated with two different kinase inhibitors (a MAPK-inhibitor and a cGMP-dependent protein kinase (PKG) inhibitor) and an inhibitor of soluble guanylyl cyclase (sGC). These two kinases and sGC are activated in the signaling transduction from the AT₂ receptor leading to neurite outgrowth.\(^{115,116}\) If 61 induces the same signaling cascade as Ang II when interacting with the AT₂ receptor, these proteins are crucial for the induction of neurite outgrowth. As shown in Figure 19 B, neurite outgrowth was inhibited when the cells were stimulated with 61 in combination with the inhibitors,
analogous to the effects seen from Ang II. Based on the results for the neurite outgrowth assay, 61 was identified to act as an agonist at the AT$_2$ receptor.

Figure 19. Effect of compound 61 on neurite outgrowth in NG108-15 cells. (A) Control, Ang II stimulation (100 nM), Ang II (100 nM) in combination with PD 123,319 (1 μM), 61 (100 nM), 61 (100 nM) in combination with PD 123,319 (1 μM). (B) Control, Ang II stimulation (100 nM), Ang II (100 nM) in combination with PD 98,059 (10 μM, MAPK-inhibition), Ang II (100 nM) in combination with LY-83,583 (500 nM, sGC-inhibition), Ang II (100 nM) in combination with KT 5823 (1 μM, PKG-inhibition), 61 (100 nM), 61 (100 nM) in combination with PD 98,059 (10 μM, MAPK-inhibition), 61 (100 nM) in combination with LY-83,583 (500 nM, sGC-inhibition), 61 (100 nM) in combination with KT 5823 (1 μM, PKG-inhibition).
Figure 20. Pictures showing the morphology of NG108-15 cells after the various conditions tested, control (A), 100 nM 61 (B and D), 100 nM Ang II (C), 1 μM PD 123,319 (E) and 100 nM 61 in combination with 1 μM PD 123,319 (F). Pictures A, B, E and F are seen at the same magnification, while C and D are seen at a higher magnification where D is a magnification of B.

6.3.2.2 Carbonate Secretion Assay

Compound 61 was also evaluated for agonistic activity at the AT₂ receptor in the duodenal mucosal alkaline secretion assay, as previously described. Intravenous administration of 61 increased the duodenal carbonate secretion in a dose-dependent manner. At the highest dose of 61, secretion was inhibited and reduced to normal by the antagonist PD 123,319, Figure 21. Topical administration of 61 into the intraluminal perfusate also increased the carbonate secretion, and coadministration with PD 123,319 reduced the effect (data not shown here). The results from the in vivo assay also strongly indicate that 61 acts as an agonist at the AT₂ receptor.

To the best of our knowledge, 61 is the first example of a selective, non-peptide AT₂ receptor agonist.
Figure 21. (A) The in vivo effect of increasing intravenous doses of 61 on rat duodenal mucosal alkaline secretion. (B) The secretory response to 61 (0.3 mg/kg h) was significantly inhibited by the AT2 selective antagonist PD 123,319 (i.v. bolus, 0.3 mg/kg h). The data is expressed as the percent change from baseline and are plotted as mean ± SEM, n = 5 in each 61 group and n = 4 in the NaCl controls (A and B).

6.4 Comparison of 61 with Pseudopeptide B

Compound 61 shares several structural features with the C-terminal of Ang II and it seems reasonable to believe that 61 mimics His\(^6\)-Pro\(^7\)-Phe\(^8\) or His\(^6\)-Pro\(^7\)-Ile\(^8\) in Ang II and its Ile\(^8\) analogues. This similarity inspired the design of pseudopeptide B, discussed in section 2.3.2.\(^{134}\) Figure 22 shows the structural features in 61, pseudopeptide B and the C-terminal amino acids in Ang II that could render similar interactions with the AT\(_2\) receptor, the imidazole ring, the acidic groups and the lipophilic side chains.\(^{134}\) Even though the design of pseudopeptide B was inspired by the identification of compound 61, it originates from peptide ligand design strategy. Consequently, both the nonpeptide ligand design strategy and the peptide ligand design strategy have identified very similar drug-like molecules selective for the AT\(_2\) receptor.
Figure 22. The imidazole rings, acidic groups and lipophilic side chains are similar structural features found in the C-terminal of Ang II, pseudopeptide B and compound 61.85

6.5 Effect of 61 on Heart Function After Myocardial Infarction

The potential of the AT2 receptor as a drug target has not yet been evaluated, due to the lack of selective drug-like agonists. We believe that 61 will be a valuable tool in this evaluation. Compound 61 has, in a preliminary study, shown beneficial effects on cardiac hemodynamics after myocardial infarction in rats. This study indicates that selective stimulation of the AT2 receptor induces tissue protective effects. Further investigations of these interesting findings are, however, required.35
7 Benzamide Structure–Activity Relationships (Paper III)

7.1 Design

After the development of 61 (section 6), the arylbenzylimidazole SAR was investigated with modifications in the 2- and 5-positions of the thiophene. The modifications had influence on AT₂ receptor affinity, but none of the compounds showed any affinity to the AT₁ receptor.¹³⁵ The N-benzylimidazole fragment was thus postulated to be a strong determinant for AT₂ receptor selectivity.¹³⁵ Nitrogen-containing heterocycles are found in most of the nonpeptide AT₁ receptor antagonists.³¹ One of the exceptions is valsartan, which has, compared to losartan, a tertiary amide instead of the imidazole, but retains the good binding to the AT₁ receptor.¹³⁶ See Figure 10 in section 2.4.1 for the structures of losartan and valsartan. Inspired by this modification, and the association of imidazoles with inhibition of the CYP450 system, two series of compounds with tertiary and secondary amides instead of the imidazole in 61 were synthesized.¹³⁷ Analogues with both the nitrogen in the same position as the alkylated nitrogen in the imidazole (valsartan-like, compound 69), as well as the inverted amide analogues (compound 70) were investigated (Figure 23). These series proved to possess high selectivity for the AT₂ receptor and include compounds with good affinity and agonistic activity. The trend in both series was that smaller substituents increased AT₂ receptor affinity.¹³⁷

\[ \text{Figure 23. The structure and biological activities of 61 and the amide analogues, 69 and 70.} \]
Guided by the trend from the amide series, where small substituents increased the AT₂ receptor affinity, we designed a series of benzamides according to the general structure of series E in Figure 24. We hypothesized that these smaller benzamides would possess high AT₂ receptor affinities in analogy to the SAR in the previous amides. Furthermore, we also designed a second series where the biphenyl and the thienylphenyl scaffolds were compared and the impact of the isobutyl substituent and its position on the AT₁/AT₂ receptor selectivity was examined (series F in Figure 24). These two series were a continuation of our SAR investigation of selective nonpeptide AT₂ receptor agonists.

Figure 24. The general structures for the synthesized compounds in series E and F.

7.2 Synthesis

The synthesis of series E is outlined in Scheme 8, and started with a Suzuki coupling of the thiopheneboronic acid 3 with 4-iodobenzoic acid. The reaction was performed under microwave irradiation, 110 °C during 5 min, with Pd(PPh₃)₄ as catalyst and Na₂CO₃ as base. The carboxylic acid 71 was isolated by extraction and transformed into the corresponding acyl chloride 72 by treatment with oxalyl chloride and a catalytic amount of DMF. The acyl chloride 72 allowed the preparation of series E to be performed in a parallel manner. Compound 72 was dissolved in dry DCM and distributed to sample vials loaded with different amines. The resulting amides were deprotected with BCl₃ or TFA, as previously described, and subsequently acylated with n-butyl chloroformate in DCM and TEA to afford the final compounds 73–84 in low to moderate yields after purification by chromatotron or preparative HPLC.

The synthesis of the compounds in series F started from a set of different benzenesulfonamides and is outlined in Schemes 9 and 10. The 3- and 4-alkylated benzenesulfonamides 87–90 were prepared from 3- and 4-bromobenzenesulfonyl chloride, starting with the conversion to the corresponding N-tert-butylsulfonamides, 85 and 86. To achieve the alkylated
analogues, compounds 85 and 86 were reacted under Negishi coupling conditions with freshly prepared isobutylzinc bromide or benzylzinc chloride,

Scheme 8.

LiBr and with (1,3-diisopropylimidazol-2-ylidene)(3-chloropyridyl)-palladium(II) dichloride (PEPPSI) as catalyst. The Negishi coupling proceeded well and provided compounds 87–90 in good to excellent yields (77–95%) (Scheme 9). Benzenesulfonamide, 4-phenylbenzenesulfonamide and phenoxybenzenesulfonamide (91–93) were prepared from their corresponding sulfonyl chlorides by treatment with tert-butylamine.
Scheme 9.

To proceed with the synthesis of series F the benzenesulfonamides, 87–93, were transformed to the corresponding boronic acids (Scheme 10). Treatment with \( n \)-BuLi gave selective ortho lithiation and the formed carbanion could subsequently be trapped by isopropylborate, and the boronic acids were achieved after acidic hydrolysis. In the boronic acid formation of 88 and 90 two position isomers could be expected to form, since the compounds are not symmetric and there are two available ortho positions to the sulfonamide. However, only one product was formed (compounds 96 and 98 respectively), which was probably due to steric hindrance. 4-iodo-(\( N,N \)-diethyl)benzenamide (94) was synthesized from para-iodobenzoic acid following the same procedure as in the amide formation in series E, thus forming the acyl chloride by reaction with oxalyl chloride followed by amidation with diethylamine. Compound 94 was coupled with the boronic acids 95–101 under the same Suzuki coupling conditions as in series E, to provide the compounds 102–108. The final products 109–115 were achieved after deprotection with BCl\(_3\) and acylation with \( n \)-butyl chloroformate, as described above, and isolated in low to good yields by column chromatography or preparative HPLC.
7.3 Biological Evaluation

7.3.1 Binding Affinity to the AT$_1$ and the AT$_2$ Receptors

Compounds 73–84 and 109–115 were evaluated for affinity to the AT$_1$ and the AT$_2$ receptors, as described in section 4.1, and the $K_i$ values are presented in Table 5 and 6.

Analogous to our hypothesis, the benzamides in series E generally possessed a higher affinity to the AT$_2$ receptor as compared to the previously
Table 5. In vitro binding affinities of the compounds in series E.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>$K_i$ (nM)</th>
<th>$\text{AT}_2$</th>
<th>$\text{AT}_1$</th>
</tr>
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<tbody>
<tr>
<td>73</td>
<td>$\text{NH}_2$</td>
<td>106 ± 0.7</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>$\text{N}$</td>
<td>36.9 ± 1.8</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>$\text{N}$</td>
<td>3.0 ± 0.3</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>$\text{H}$</td>
<td>2.6 ± 0.2</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>$\text{N}$</td>
<td>10.5 ± 0.5</td>
<td>&gt;10,000</td>
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<tr>
<td>78</td>
<td>$\text{N}$</td>
<td>6.4 ± 0.5</td>
<td>&gt;10,000</td>
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<tr>
<td>79</td>
<td>$\text{N}$</td>
<td>1.0 ± 0.08</td>
<td>&gt;10,000</td>
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<tr>
<td>80</td>
<td>$\text{N}$</td>
<td>31.5 ± 0.7</td>
<td>&gt;10,000</td>
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</tr>
<tr>
<td>81</td>
<td>$\text{N}$</td>
<td>10.9 ± 0.4</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>$\text{N}$</td>
<td>6.4 ± 0.3</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>$\text{N}$</td>
<td>3.2 ± 0.2</td>
<td>&gt;10,000</td>
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</tr>
<tr>
<td>84</td>
<td>$\text{N}$</td>
<td>348 ± 5.4</td>
<td>&gt;10,000</td>
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</table>
evaluated N-benzylamides and phenylacetamides. All compounds exhibited high selectivity for, and moderate to high affinity to, the AT$_2$ receptor (Table 5). The primary benzamide 73 only possessed a moderate affinity of 106 nM. The introduction of methyl substituents in 74 substantially increased the affinity to 36.9 nM, but it was still lower than the corresponding N,N-dimethylphenylacetamide, 70 (7.0 nM), which was the most potent phenylacetamide. Extending the substituents one additional carbon, as in 75, resulted in an affinity of 3.0 nM, which was better than 70. Compound 75 was the most potent tertiary benzamide. The related piperidino analogue 83 was equipotent compared to 75, while the pyrrolidino analogue 82 showed slightly lower affinity. Notably, when exchanging the piperidino in 83 to the morpholino in 84, the affinity dropped dramatically. The introduction of oxygen in this position seems to impair the interaction with the AT$_2$ receptor. Among the secondary amides, the benzyl substituent in 79 possessed the highest affinity, 1.0 nM, which was the most potent compound in the series E. Interestingly, the N,N-benzylmethylbenzamide analogue 80 exhibited a 30 times lower affinity compared to 79. The cyclopropyl and the tiazolyl benzamides (77 and 81 respectively) exhibited similar affinities, while the isopropyl analogue showed five times higher affinity. The cyclohexyl analogue 78 was equipotent to the pyrrolidino, 82.

From series F it was obvious that the thiarylphenyl scaffold is not exchangeable to the biphenyl scaffold in N,N-diethylbenzamides (Table 6). It has previously been shown that these two scaffolds are bioisosteric in this type of structures, for example in the balanced AT$_1$/AT$_2$ ligands L-162,313 and L-162,782 (see Figure 5). We have previously shown that this is also true for 61, its biphenyl analogue exhibits equipotent affinity. In this series the biphenyl compounds 109 and 110 lose 50 times in affinity, as compared to the thiarylphenyl analogue 75. Notably, there was no clear difference between the two positions of the isobutyl substituent. Compound 110, with the isobutyl substituent in the 5-position, showed slightly better affinity compared to 109, with the isobutyl substituent in the 4-position. In the N,N-diethylbenzamides it is clear that the isobutyl in neither the 4- nor in the 5-position of the biphenyl can reach the same favourable interaction with the AT$_2$ receptor as the isobutyl in the 5-position of the thiarylphenyl scaffold. On the other hand it was evident that this lipophilic substituent is important for good affinity to the AT$_2$ receptor, since compound 113, lacking this substituent, exhibited a much lower affinity to the AT$_2$ receptor (402 nM) compared to both 75 (3.0 nM) and 109 (144.5 nM).

The benzyl substituted analogues, 111 and 112, possessed a lower affinity to the AT$_2$ receptor, compared to the isobutyl substituted analogues. With this larger substituent the difference between the two positions was more pronounced, favouring the substituent in the 4-position (111). Compound 115, with a phenoxy substituent instead of the isobutyl showed a higher affinity compared to both 109 and 111, but the highest affinity in this series
was surprisingly achieved by the triphenyl, 114. The very rigid analogue 114, with a phenyl instead of the isobutyl, exhibited an affinity of 52.0 nM. The phenyl must thus induce a more favourable interaction with the AT\textsubscript{2} receptor than both the isobutyl substituted analogues 109 and 110.

Table 6. In vitro binding affinities of the compounds in series F

<table>
<thead>
<tr>
<th>Compound</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>K\textsubscript{1} (nM)</th>
<th>AT\textsubscript{2}</th>
<th>AT\textsubscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>109</td>
<td>(\text{isobutyl})</td>
<td>H</td>
<td>145 ± 3.4</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>H</td>
<td>(\text{isobutyl})</td>
<td>122 ± 1.8</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>(\text{isobutyl})</td>
<td>H</td>
<td>163 ± 1.0</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>H</td>
<td>(\text{phenyl})</td>
<td>300 ± 2.9</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>H</td>
<td>H</td>
<td>402 ± 10.1</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>(\text{phenyl})</td>
<td>H</td>
<td>52.0 ± 4.3</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>(\text{phenyl})</td>
<td>H</td>
<td>91.7 ± 2.3</td>
<td>&gt;10,000</td>
<td></td>
</tr>
</tbody>
</table>

In series F, apart from comparing the two scaffolds, we wanted to investigate the impact of the isobutyl substituent and its position on the AT\textsubscript{1}/AT\textsubscript{2} receptor selectivity. To our surprise, none of the compounds possessed any affinity towards the AT\textsubscript{1} receptor. It has previously been shown that modification of the size of the isobutyl substituent increases AT\textsubscript{1} receptor affinity.
but decreases AT$_2$ receptor affinity.\textsuperscript{106,107,110} However, according to series F, these modifications do not seem to influence on the selectivity pattern. Thus, in these smaller nonpeptidic AT$_2$ receptor ligands there seems to be a strong discrimination between the receptor subtypes. So far we have not encountered any AT$_1$ receptor affinity since we left the bicyclic heteroaromatic head of the structures. Consequently, both the smaller heteroaromatic and amide-based substituents are strong determinants for AT$_2$ receptor selectivity.

### 7.3.2 Functional Activity at the AT$_2$ Receptor

Three compounds were selected for evaluation of functionality at the AT$_2$ receptor in the neurite outgrowth assay in NG108-15 cells, as described in section 4.2.1. From series E, 75 and 79 were selected and from series F the triphenyl, 114 was selected. All three compounds induced neurite outgrowth in an extent comparable to Ang II (Figure 25). Compounds 75 and 79 were tested at two concentrations, 1 and 10 nM. Both concentrations induced neurite outgrowth, and the higher concentration gave a slightly higher degree of neurite outgrowth (data not shown). Compound 114 needed a higher concentration to induce neurites to the same extent. Coincubation with the selective AT$_2$ receptor antagonist PD 123,319 reduced the neurite outgrowth to control levels, verifying an effect via the AT$_2$ receptor (Figure 25). All three compounds that were evaluated for agonism or antagonism at the AT$_2$ receptor were therefore identified as agonists. These SAR investigations have led us to potent, highly selective, nonpeptide AT$_2$ receptor agonists devoid of the imidazole ring that is associated with CYP450 inhibition.

![Figure 25. Effect of 75, 79 and 114 on neurite outgrowth in NG108-15 cells. White: Untreated control cells. Black: Cells treated with Ang II (100 nM), 75 (1 nM), 79 (1 nM), or 114 (100 nM) alone. Light grey: Cells treated with Ang II (100 nM), 75 (1 nM), 79 (1 nM), or 114 (100 nM) in combination with PD 123,319 (1 μM).](image)
8 Interconversion of Functional Activity by Minor Structural Alterations (Paper IV)

8.1 Design

In our continuing SAR investigation of selective nonpeptide AT₂ receptor agonists, compound 116, the \textit{meta} substituted analogue of 61 (section 6), was synthesized (Figure 26, manuscript in preparation, Murugaiah \textit{et al.}). Compound 116 showed high selectivity and good affinity to the AT₂ receptor and was selected for evaluation in the neurite outgrowth assay for functional activity. In this evaluation the \textit{meta} analogue 116 was found to act as an antagonist at the AT₂ receptor (Figure 26). Compound 116 did not induce neurite outgrowth alone and Ang II-induced neurite outgrowth was inhibited by coincubation with 116 (Figure 27, see Appendix, section 12, for the experimental procedure of the evaluation of 116 in the neurite outgrowth assay). Consequently, by moving the methylenimidazole from the \textit{para} position to the \textit{meta} position in 61, a selective nonpeptide AT₂ receptor antagonist was achieved. Thus, this minor structural alteration leads to an interconversion of the functionality in these nonpeptide AT₂ ligands. As mentioned in section 1.3.3, there are more examples of nonpeptide agonists to peptide-activated GPCRs where minor structural modifications lead to

\[ \text{Figure 26. The structure and biological activities of the selective nonpeptide AT}_2 \text{ receptor agonist } 61, \text{ and the } \text{meta} \text{ substituted analogue } 116, \text{ which acts as an antagonist at the AT}_2 \text{ receptor.} \]
interconversion of functionality. The most related example to our project is the non-peptide AT\textsubscript{1} receptor ligands L-162,782 and L-162,389 (see Figure 5, section 2.4.4 for structures). These two structures differ only by one methyl group – the isobutyl substituent in L-162,782 renders agonistic properties while the \textit{n}-propyl substituent in L-162,389 renders antagonistic properties.

The only structural difference between the agonist 61 and the antagonist 116 is the position of the methyleneimidazole substituent. Consequently, the change in functionality could result from a reduced ability of 116 to stabilize the activated conformation of the receptor, due to the differently positioned imidazole ring. However, our attention was drawn to an alternative hypothesis that arose based on molecular modeling. This hypothesis showed that the imidazole rings in 61 and 116 may occupy the same space, while the isobutyl groups, on the other hand, were oriented differently. Thus, differently oriented isobutyl groups during binding could also be the explanation for the change in functionality. The alternative hypothesis is illustrated in Figure 28 A, where possible pharmacophore groups in 61 and 116, including the imidazole rings, are superimposed using the pharmacophore modeling program DISCOtech in Sybyl 7.3. Based on this possible binding mode, we hypothesized that the \textit{meta} substituted antagonist, 116, could be converted to an agonist if the isobutyl group could adopt a similar orientation as in the \textit{para} substituted agonist, 61 (Figure 28 A). This movement of the isobutyl group in 116 could render a similar binding mode as that of the agonist 61. To explore this hypothesis and to investigate the structural requirements for agonists and antagonists, we designed a series of biphenyl analogues where the relative positions of the methyleneimidazole and the isobutyl substituent were varied (117, 123–125, Figure 29 A). Even though the biphenyl
Figure 28. (A) Possible binding mode of the two thienylphenyl structures, 61 (white) and 116 (green), the movement of the isobutyl group in 116 to the position of the isobutyl group in 61 could regain agonistic activity in 116. (B) Corresponding binding mode found for the biphenyl analogues, 117 (purple) and 123 (orange) and the postulated functions at the AT₂ receptor. (C) Comparison of 61 and 116 with the analogues of 117 and 123, where the isobutyl group has been moved to the meta position, 124 (red) and 125 (blue) respectively.

scaffold is not a general bioisostere to the thienylphenyl scaffold in selective AT₂ receptor ligands (see section 7.3.1), it has been proven to render the same affinity with the methyleneimidazole substituent. The biphenyl 117 has previously been synthesized and evaluated for affinity to the AT₁ and the AT₂ receptors (AT₂: \( K_i = 0.7 \text{ nM}, \text{AT}_1: K_i >10,000 \text{ nM} \)). As a first step we superimposed the biphenyl analogues of 61 and 116, compounds 117 and 123. The model is shown in Figure 28 B. The model of 117 and 123 was very similar to the model achieved from 61 and 116 (Figure 28 A and B). We also modelled the corresponding analogues with the isobutyl group in the meta position to the sulfonylcarbamate, compounds 124 and 125, and compared their binding modes with those suggested for 61 and 116 (Figure 28 C). According to our hypothesis discussed above, 125 was postulated to act as an agonist. Even though the isobutyl group in 125 did not reach the exact same position as the isobutyl group in 61, the imidazole and the sulfonylcarbamate substituents stayed in a position where they could reach the same interactions (Figure 28 C). Compound 124 was also postulated to act as
an agonist since it showed a very similar binding mode compared to 125 (Figure 28 C). Compound 117 has not been evaluated for functional activity, which would give further information on the true bioisosteric properties of the biphenyl scaffold compared to the thienylphenyl scaffold. Compounds 123–125 were synthesized and all compounds were biologically evaluated (Figure 29 A).

**Figure 29.** (A) The biphenyl derivatives, 117, 123–125. (B) The corresponding analogues with rigified isobutyl groups, 148a/b, 149a/b, 153a/b and 154a/b (a and b denotes the two enantiomers).

Furthermore, to get more detailed information about the impact of the direction of the isobutyl group on functionality of AT2 receptor ligands, we synthesized and evaluated the corresponding analogues with conformationally constrained isobutyl groups as pure enantiomers, 148a/b, 149a/b, 153a/b and 154a/b, where a and b denotes the two enantiomers (Figure 29 B). With these analogues we aimed to probe the bioactive conformation(s) of nonpeptide AT2 receptor agonists and therefore all of these rigidified analogues were evaluated both for affinity to, and functional activity at, the AT2 receptor.
8.2 Synthesis

The synthesis of the biphenyl analogues 123–125 is outlined in Scheme 11, and started with the benzenesulfonamides 87 and 88. Compounds 87 and 88 were transformed into the corresponding boronic acids 95 and 96, as described in section 7.2 (Scheme 10). The aryl bromides 118 and 119 were afforded by N-alkylation of imidazole with 4-bromobenzyl bromide. Compounds 118 and 119 were coupled with the crude boronic acids 95 and 96.
in Suzuki coupling reactions, with Pd(PPh₃)₄ as palladium source and Na₂CO₃ as base under microwave irradiation (150 °C, 5 min) to form the intermediates 120–122 in good yields (75–89%). The final products 123–125 were achieved after deprotection with BCl₃ in DCM and subsequent acylation with n-butyl chloroformate in the two-phased system DCM and water, with Na₂CO₃ as base.

Scheme 12.

For the analogues with rigified isobutyl substituents we chose to use an indan scaffold. Before synthesizing compounds 148a/b, 149a/b, 153a/b and 154a/b, we wanted to verify that the extra carbons introduced with the indan scaffold did not interrupt the interaction with the AT₂ receptor. Therefore we first prepared the corresponding unsubstituted indan analogues, 131, 132, 135 and 136 (Scheme 12). The synthesis started with the formation of the
sulfonamide 126 from indan as outlined in Scheme 12. Compound 126 was then transformed to the boronic acids 127 and 128, using the same procedure as described in section 7.2. Contrary to the boronation of 88 and 90, compound 126 yielded two boronic acids, which is probably due to a lower degree of steric hindrance in the bicyclic indan structure. The crude mixture of the boronic acids 127 and 128 were coupled with 118 and 119 in Suzuki coupling reactions, as described above, to form compounds 129, 130, 133 and 134. The products were separated using preparative HPLC. The isolated compounds were then deprotected and acylated, as described above, to form the final products 131, 132, 135 and 136. For experimental data on compounds 126–136 see Appendix, section 12. Compounds 130, 132 and 135 are only characterized by 1H-NMR.

Scheme 13.

In the synthesis of the rigified analogues, 1-indanone was used as starting material and the synthesis is outlined in Schemes 13, 14 and 15. The first step was the formation of 1-isopropylindan, 137, which was prepared essentially as described by Phillips et al. (Scheme 13). Thus, 1-indanone was converted to 1-isopropylindan in a Grignard reaction with freshly prepared
2-propylmagnesium bromide. The unsaturated compound was achieved directly after the acidic work up due to a spontaneous elimination of the benzyl alcohol group, and used without further purification. Compound 137 was afforded after hydrogenation with PtO2 as catalyst. The two sulfonyl chloride position isomers 138 and 139, were formed after treatment of 137 with chlorosulfonic acid. The two isomers were formed in approximately equal amounts and they were separated by column chromatography. Compounds 138 and 139 were then directly reacted with tert-butylamine to form the protected sulfonamides 140 and 141. The separation had to be performed on the sulfonyl chlorides since the N-tert-butyl protected sulfonamides 140 and 141 were unable to separate on silica. The racemic mixtures of 140 and 141 were separated using preparative chiral HPLC to isolate the pure enantiomers 140a/b and 141a/b (Scheme 13). Chiral GC–MS was used to verify the enantiomeric purity of isolated enantiomers, which proved to be 99% for all enantiomers. The pure enantiomers were transformed into the corresponding boronic acids, using the procedure described above (Schemes 14 and 15). Compounds 140a and 140b formed two boronic acids each, 142a/143a and 142b/143b respectively, in analogy with the formation of boronic acids 127 and 128 (Scheme 14). Compounds 141a and 141b, on the other hand, only afforded one boronic acid each, 150a and 150b respectively, probably due to the steric hindrance induced by the isopropyl group (Scheme 15). The crude boronic acids were coupled with 118 and 119 in Suzuki coupling reactions, as described above, to form the intermediates 144a/b, 145a/b, 146a/b, 147a/b, 151a/b and 152a/b. Compounds 144a/b and 145a/b were isolated by preparative HPLC from the product mixtures after the Suzuki coupling of boronic acids 142a/143a and 142b/143b with 118 and 119. Unfortunately, we did not succeed in isolating compounds 146a/b and 147a/b from this product mixture. Compounds 151a/b and 152a/b were also purified by preparative HPLC. The final products 148a/b, 149a/b, 153a/b and 154a/b were afforded after the final step with deprotection and acylation of the primary sulfonamides, as previously described. All final products were purified by preparative HPLC and achieved in 48–61% yield.
Scheme 14.

\[
\begin{align*}
140a \ (\text{+}), \ 140b \ (-) \\
\xrightarrow{1) \ n-BuLi \ 2) \ \text{B(OH)}_2 \ (\text{Pr}_3) \ 3) \ \text{HCl (aq)}} \text{THF} \\
142a, 142b \\
\text{118, 119} \\
\xrightarrow{\text{Pd(PPh}_3)_2} \text{Na}_2\text{CO}_3 \ (\text{aq}) \text{toluene, ethanol, MW} 150 \degree \text{C, 5 min}} \\
144a, 144b \ (\text{+}), 145a, 145b \ (\text{+}) \\
\text{146a, 146b, 147a, 147b} \\
\text{144a (\text{+}), 144b (\text{+}), 145a (\text{+}), 145b (\text{+})} \\
\xrightarrow{\text{n-butyl chloroformate, Na}_2\text{CO}_3} \\
148a, 148b \ (\text{+}), 149a, 149b \ (\text{+})
\end{align*}
\]
8.3 Biological Evaluation

All final products, 123–125, 131, 132, 135, 136, 148a/b, 149a/b, 153a/b and 154a/b, were evaluated for affinity to the AT$_2$ receptor, as described in section 4.1. Compounds 123–125 were not evaluated for AT$_1$ receptor affinity, since we have not seen any affinity to the AT$_1$ receptor when modifying the lower part of 61.$^{135}$ The unsubstituted indan analogues, compounds 131, 132, 135 and 136, were evaluated for both AT$_1$ and AT$_2$ receptor affinity to investigate the effect of the new indan scaffold. None of the compounds possessed affinity to the AT$_1$ receptor, so we chose to focus the evaluation of the rigi-fied enantiomers on AT$_2$ receptor affinity. Compounds 117, 123–125, 148a/b, 149a/b, 153a/b and 154a/b were evaluated in the neurite outgrowth assay for functional activity at the AT$_2$ receptor. $K_i$ values and functional responses are summarized in Figures 30, 32 and 33.
The biphenyl analogue of 61, compound 117, has already been evaluated for affinity to the AT$_2$ receptor and it proved to be approximately equipotent to 61, with an affinity of 0.7 nM as compared to 0.4 nM (Figure 30). In the neurite outgrowth assay 117 also proved to function as an agonist at the AT$_2$ receptor, as its thienylphenyl analogue 61 (Figure 31). The biphenyl analogue of the antagonist 116 (Figure 26), compound 123 showed a slightly higher affinity, 12.3 nM compared to 17.8 nM, and retained the antagonistic property (Figure 30). Compound 123 did not induce neurite outgrowth alone and inhibited the Ang II-induced neurite outgrowth, as can be seen in Figure 31. These results imply that the biphenyl scaffold is a bioisostere to the thienylphenyl scaffold in these compounds, since both affinities and functions were retained in 117 and 123. The corresponding analogues of 117 and 123, with the isobutyl group positioned meta to the sulfonylecarbamate, 124 and 125 respectively, exhibited good affinities to the AT$_2$ receptor. Compound 124 was well tolerated by the receptor and possessed an affinity of 10.1 nM, while compound 125 exhibited twice as high affinity as 116 (Figure 30). Notably, both compounds acted as agonists at the AT$_2$ receptor, as can be seen in Figure 31. Thus, in agreement with our hypothesis, the antagonistic compound 123, could be converted to an agonist by moving the isobutyl side chain from the para to the meta position on the lower phenyl.
Figure 31. Effect of compounds 117, 123–125 on neurite outgrowth in NG108-15 cells. White: Untreated control cells. Black: Cells treated with Ang II (100 nM), 117 (10 nM), 123 (10 nM), 124 (10 nM) or 125 (10 nM) alone. Light grey: Cells treated with Ang II (100 nM), 117 (10 nM), 123 (10 nM), 124 (10 nM) or 125 (10 nM) in combination with 1 μM PD 123,319. Dark grey: Cells treated with 117 (10 nM), 123 (10 nM), 124 (10 nM) or 125 (10 nM) in combination with Ang II (100 nM).

ring, rendering 125. Since the agonistic compound 125 has the methylneimidazole in the meta position, the structural feature responsible for functional activity cannot only be the position of the imidazole substituent. Instead, these results suggest that it is the spatial relationship between the imidazole ring and the isobutyl group that determines the functional activity. Compounds 124 and 125 could, according to the model in Figure 28 C, possess similar binding modes. The relationship between the imidazoles resembles that of 61 and 116 in Figure 28 A, while the isobutyl groups in 124 and 125 are more overlapping. These two compounds seem to be able to adopt a similar spatial relationship between their imidazole rings and isobutyl substituents that renders agonism. Even though the relationship is not exactly the same as in 61, both compounds act as agonists. The same function can
thus be achieved by two compounds that differ in the position of the imidazole substituent.

Figure 32. In vitro binding affinities of compounds 131, 132, 135 and 136.

The $K_i$ values of the unsubstituted indan analogues, compounds 131, 132, 135 and 136, are shown in Figure 32. None of the compounds possessed affinity to the AT$_1$ receptor. The indan scaffold did not interrupt the interaction with the AT$_2$ receptor and the compounds exhibited moderate affinities. Even though it seems like the extra volume is tolerated by the receptor, it does not replace the isobutyl group. Interestingly, the impact of the relative positions on affinity was pronounced, and the relationships were reversed when comparing the para substituted compounds, 131 and 132 with the meta substituted compounds, 135 and 136. This effect could imply that the relationship between the imidazole and the alkyl substituent is important and that the loss, when moving one of them, can be compensated for by moving the other to restore the relationship. Compounds 131 and 136 possessed the highest affinities in the respective pairs (Figure 32).

The alkylated indan analogues with conformationally constrained isobutyl chains exhibited generally higher affinities to the AT$_2$ receptor as compared to the unsubstituted analogues (Figure 33). Thus, the alkyl group increases the affinity. In the case of biphenyl analogues 124 and 125, with the isobutyl substituent in the meta position to the sulfonylcarbamate, the rigidified analogues 153a/b and 154a/b respectively, showed improved affinity.
Figure 33. In vitro binding affinity and functional activity of compounds 148a/b, 149a/b, 153a/b and 154a/b. (Figures 30 and 33). The higher affinity implies that the rigid analogues have an improved fit in the receptor and/or a lower entropy loss during binding to the AT2 receptor. The opposite effect was seen in the case of the biphenyl analogues 117 and 123, with the isobutyl substituent in the para position to the sulfonylcarbamate. Here the rigidified analogues 148a/b and 149a/b showed a decreased affinity as can be seen when comparing Figures 30 and 33. Interestingly, when the enantiomers were evaluated for functional activity at the AT2 receptor the functionality was not consistent within the enantiomeric pair in two of the cases, 148a/b and 154a/b, as can be seen in Figures 34 and 35. In both cases the nonrigidified biphenyl analogue acted as agonists, compounds 117 and 125 respectively. Compound 148a lost nearly 20 times in affinity compared to 117, but it retained the agonistic activity and induced the highest degree of neurite outgrowth in this set of compounds (Figure 34). The enantiomer, 148b, retained more of the affinity but acted as an antagonist at the AT2 receptor. Compound 148b, with a $K_i$ of 2.8 nM, is
so far the antagonist with the highest affinity in this compound class. Compound 154a also acted as an antagonist with low nanomolar affinity, while the enantiomer 154b retained the agonistic property from the nonrigidified compound 125 (Figure 35). These results suggest that the functional response is sensitive to the direction of the isobutyl substituent and that the rigidified analogues 148b and 154a are locked out from the bioactive conformation(s). Even though the structural difference between these enantiomers is very small, it has a big impact on the ability to stabilize the active conformation of the AT2 receptor. The rigidified analogues of 123 and 124, 149a/b, and 153a/b retained the agonistic and antagonistic properties, respectively. Thus, there is a difference in the sensitivity to the direction of the isobutyl group between the analogues. Compounds 117 and 125 seem to possess spatial relationships that are sensitive to the direction of the isobutyl substituent for
functional activity. Compounds 123 and 124, on the other hand, seem to possess spatial relationships that show no difference in functionality depending on direction of the isobutyl substituent.

Figure 35. Effect of compounds 153a/b and 154a/b on neurite outgrowth in NG108-15 cells. White: Untreated control cells. Black: Cells treated with Ang II (100 nM), 153a (10 nM), 153b (10 nM), 154a (10 nM) or 154b (10 nM) alone. Light grey: Cells treated with Ang II (100 nM), 153a (10 nM), 153b (10 nM), 154a (10 nM) or 154b (10 nM) in combination with 1 μM PD 123,319. Dark grey: Cells treated with 153a (10 nM), 153b (10 nM), 154a (10 nM) or 154b (10 nM) in combination with Ang II (100 nM).

Figure 36 shows an overview of the starting thienylphenyl structures and the investigated biphenyl structures, including their affinity to and functional activity at the AT2 receptor.

At this stage it is difficult to rationalize the results from the functionality evaluation of the enantiomers. In the future, when crystallization of GPCRs is more accessible, it would be interesting to see the two pairs of enantiomeric agonists and antagonists, 148a/b and 154a/b, in a crystal structure of the AT2 receptor. With the help of crystal structures it might be possible to understand why these small structural differences have such a big impact on
functional activity. Our results from the rigidified analogues have not fully cleared the picture of how the bioactive conformation(s) look. To investigate this further, more constrained analogues are required. However, compounds 148a, 154b and 153a/b have given us valuable information regarding the conformations of the isobutyl group in nonpeptide AT_2 receptor agonists.

**Figure 36.** Overview of reference structures (61 and 116) and the investigated biphenyl compounds including their respective affinity to and functional activity at the AT_2 receptor.
9 Conclusions

The present study has led to the discovery of the first selective nonpeptide \textit{AT\textsubscript{2}} receptor agonist and contributed to a better understanding of the SAR of nonpeptide \textit{AT\textsubscript{2}} receptor agonists and antagonists, as summarized below.

- The nonselective (\textit{AT\textsubscript{1}}/\textit{AT\textsubscript{2}}) compound L-162,313 acts as an agonist at both the \textit{AT\textsubscript{1}} and the \textit{AT\textsubscript{2}} receptors.

- A stepwise modification of the imidazopyridine in L-162,313 led to the identification of the first selective nonpeptide \textit{AT\textsubscript{2}} receptor agonist, compound 61.

- In the SAR investigation of selective nonpeptide \textit{AT\textsubscript{2}} receptor agonists, new leads, where the benzylimidazole has been replaced with substituted benzamides, have been identified, as exemplified by the agonist 79.

- From the neurite outgrowth assay in NG108-15 cells, it was shown that by moving the methylenimidazole in the agonist 61 from the \textit{para} to the \textit{meta} position, the selective nonpeptide \textit{AT\textsubscript{2}} receptor antagonist, compound 116, was achieved.
The structural requirement for agonistic vs antagonistic properties is not solely dependent on the position of the methyleneimidazole. The functionality seems, at least partly, to be attributed to the spatial relationship between the methyleneimidazole and the isobutyl side chain. The antagonistic compound 123 can be converted to the agonist 125 by moving the isobutyl group from the para to the meta position in the lower phenyl ring.

The biphenyl scaffold is often, but not always, bioisosteric to the thienylphenyl scaffold in the nonpeptide AT\(_2\) receptor ligand series. The biphenyl scaffold of 117 and 123 serves as a bioisostere of the thienylphenyl scaffold of 61 and 116.
The AT\textsubscript{2} receptor (active and inactive conformations) responds to the position of the isobutyl chain in space, as demonstrated by a comparison of the conformationally constrained enantiomerically pure analogues 148a/b and 154a/b. Further investigations are required to fully elucidate the bioactive conformation(s) of nonpeptide AT\textsubscript{2} receptor agonists.

148a (-)
\[K_1 (\text{nM})
\]
AT\textsubscript{2}: 127.4 ± 4.6
Agonist (AT\textsubscript{2})

148b (+)
\[K_1 (\text{nM})
\]
AT\textsubscript{2}: 2.8 ± 0.2
Agonist (AT\textsubscript{2})

154a (+)
\[K_1 (\text{nM})
\]
AT\textsubscript{2}: 3.2 ± 0.1
Agonist (AT\textsubscript{2})

154b (-)
\[K_1 (\text{nM})
\]
AT\textsubscript{2}: 7.0 ± 0.2
Agonist (AT\textsubscript{2})
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I wish to express my sincere gratitude to the following people, without whom the work on this thesis would have been much less enjoyable, and in some cases, not even possible.

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Paul, för att du alltid finns där för mig och visar vad som verkligen är viktigt i livet. ☺️
11 References


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141. SYBYL 7.3, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.

Appendix

Evaluation of compound 116 in the neurite outgrowth assay.

General considerations. The chemicals used were obtained from the following sources: Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), HAT supplement (Hypoxanthine, Aminopterin, Thymidine), gentamycin from Gibco–BRL (Burlington, Ont., Canada); [Val\textsuperscript{5}]angiotensin II from Bachem (Marina Delphen, CA, USA). PD 123,319 was obtained from RBI (Natick, MA, USA). All other chemicals were of grade A purity.

Cell culture. To study the in vitro morphological effects NG108-15 cells (provided by Drs. M. Emerit and M. Hamon; INSERM, U. 238, Paris, France) were used as well as transfected NG108-15/pcDNA3 cells. The transfected cell line has previously been shown to have the same behavior as the native cell line.\textsuperscript{117} In their undifferentiated state, neuroblastoma x glioma hybrid NG108-15 cells have a rounded shape and divide actively. Both cell lines were cultured (NG108-15 passage 18–28, NG108-15/pcDNA3 passage 12–18) in Dulbecco's modified Eagle's medium (DMEM, Gibco–BRL, Burlington, Ont., Canada) with 10% fetal bovine serum (FBS, Gibco), HAT supplement and 50 mg/L gentamycin at 37 °C in 75 cm\textsuperscript{2} Nunclon Delta flasks in a humidified atmosphere of 93% air and 7% CO\textsubscript{2}, as previously described.\textsuperscript{43,51} The transfected cell line was kept stable by addition of Geneticin (G-418, 200 g/mL) to the media.\textsuperscript{21} Subcultures were performed at subconfluency. Under these conditions, cells express only the AT\textsubscript{2} receptor subtype.\textsuperscript{43,51} Cells were treated during three days, once a day (first treatment 24 h after plating), and micrographs were taken the fourth day. For all experiments, cells were plated at the same initial density of 3.6 \times 10\textsuperscript{4} cells/35 mm Petri dish. To determine a good test concentration compound 116 was tested at various concentrations ranging from 1 pM to 1 \muM. It was only in the highest concentration that a tendency of cell death was observed. Cells were treated without (control cells), or with [Val\textsuperscript{5}]angiotensin II (100 nM) or 116 (10 and 100 nM) in the absence or in the presence of PD 123,319 (10 \muM), an AT\textsubscript{2} receptor antagonist introduced daily 30 min prior to Ang II or compound, to evaluate agonistic properties. Compound 116 (10 and 100 nM) was also tested in the presence of Ang II (100 nM) where 116 was introduced daily 30 min prior to Ang II, to evaluate antagonistic properties. During the three days treatment the transfected cell line was cultured without Geneticin.
**Determination of cells with neuritis.** Cells were examined under a phase contrast microscope and micrographs were taken after 3 days under the various experimental conditions. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites was reported as the percentage of the total amount of cells in the micrographs and at least 290 cells were counted in three independent experiments and each condition was performed in duplicate. The data are represented as means ± SEM of the average number of cells on a micrograph.

**Experimental data for compounds 126–136.**

**General Considerations.** $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR spectra were recorded on a Varian Mercury 400 at ambient temperature, if not otherwise stated. Chemical shifts are given as δ values (ppm) downfield from tetramethylsilane and referenced to δ 7.26 and δ 77.16 for CDCl$_3$ and δ 3.31 and δ 49.00 for CD$_3$OD. Analytical GC-MS with EI ionization was performed on a Varian 3800 or 3900 equipped with a CP-SIL 5 CB Low Bleed (30 m x 0.25 mm) or CP-SIL 8 CB Low Bleed (30 m x 0.25 mm), using He as carrier gas. RP-HPLC was performed on a Gilson HPLC system equipped with a Zorbax SB C8, 5 μm (4.6 x 50 mm) column and a Finnigan AQA quadrupole mass spectrometer. The mobile phase consisted of H$_2$O/ MeCN (0.05% HCOOH) and the analyses were run in a gradient mode. Preparative RP-HPLC was performed on a Gilson-Finnigan Thermo Quest AQA system equipped with a Zorbax SB-C8, 5 μm 21.2 x 150 mm (Agilent technologies) column at a flow rate of 15 mL/min. The mobile phase consisted of H$_2$O/ MeCN (0.05% HCOOH). Preparative and analytical RP-HPLC was performed on a system consisting of a Gilson 321 pump and a LKB 2151 variable wavelength detector equipped with a Zorbax SB C8 5 μm (21.2 x 150 mm) column, a ACE 5 Phenyl (150 x 21.2 mm) and a Zorbax SB C8 5 μm (4.6 x 50 mm) column, respectively. The mobile phase consisted of H$_2$O/ MeCN with 0.1% TFA or H$_2$O/ MeCN with 0.05% HCOOH. Microwave heating was performed using Emrys Initiator™ single mode cavity, producing controlled irradiation at 2450 MHz. Dedicated microwave vials from Biotage were used for the reactions and the temperature of the reaction mixture was measured using a built-in, on-line infrared temperature sensor. Exact molecular masses (HRMS) were determined on a Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. Dry CH$_2$Cl$_2$ was distilled over calcium hydride and dry THF over sodium. Other chemicals and solvents used were of analytical grade and purchased from commercial suppliers, and used without further purification unless stated (e.g. dry THF). Thin-layer chromatography was performed on aluminium plates pre-coated with silica gel 60 F$_{254}$ (Merck) and visualized with UV light. Flash chromatography was performed on silica gel 60 (0.040-0.063 mm, Merck).
6-(N-tert-Butylsulfonamido)-indan (126). Indan (2.9 g, 24.5 mmol) was dissolved in dry DCM (8.5 mL) and cooled on an icebath. Chlorosulfonic acid (14.8 g, 127 mmol) was added dropwise and the mixture was left on the icebath for 1 h and then heated to 40 °C during 1 h. The reaction mixture was poured into a stirring icebath (150 mL) and extracted with EtOAc. The organic phase was washed with water and brine and dried over MgSO₄ and evaporated. The crude product was dissolved in DCM (20 mL) and cooled on an icebath. N-tert-butylamine (9.74 g, 133 mmol) was added dropwise and the mixture was left for 30 min and then refluxed for 10 min. The reaction mixture was diluted with toluene, washed with water and brine and dried over MgSO₄ and evaporated. The crude product was purified by column chromatography (100% DCM) to afford 126 as white crystals in 63% yield. ¹H NMR (CDCl₃), δ, ppm: 7.73-7.72 (m, 1H), 7.68-7.65 (m, 1H), 7.30-7.28 (m, 1H), 4.62 (brs, 1H), 2.95 (t, J = 7.4 Hz, 4H), 2.13 (qui, J = 7.4 Hz, 2H), 1.23 (s, 9H). ¹³C NMR (CDCl₃), δ, ppm:149.3, 145.3, 141.4, 125.4, 124.7, 123.0, 54.7, 33.0, 32.8, 30.3, 25.5. Anal. Cald. for C₁₃H₁₉NO₂S: C, 61.63; H, 7.56; N, 5.53. Found: C, 61.58; H, 7.70; N, 5.48.

General procedure for compounds 129, 130, 133 and 134. To a cooled (-78 ºC) solution of compound 126 (2.52 g, 9.95 mmol) in dry THF (40 mL), was n-BuLi (1.6 M in hexane, 17 mL, 27.2 mmol) added under nitrogen atmosphere and stirred for 1 hour. The temperature was raised to -30 ºC and kept for 3 hours and subsequently decreased to -40 ºC. Triisopropyl borate (3.4 mL, 14.9 mmol) was then added. The reaction was stirred over night at room temperature. The reaction was cooled (0 ºC) and treated with an excess of 2 M HCl solution. The mixture was extracted with EtOAc and the combined organic phase was washed with water and brine. The organic layer was dried with MgSO₄, filtered and evaporated. The crude product mixture of 127 and 128 was then used in the next step without further purification. A microwave vial (2-5 mL) was charged with the crude boronic acid, compound 118 or 119, toluene, ethanol, 2 M Na₂CO₃ and Pd(PPh₃)₄. The reaction mixture was flushed with nitrogen, sealed and heated by microwave irradiation to 150 ºC for 5 min. The reaction mixture was diluted with EtOAc and water. The water phase was extracted with EtOAc and the combined organic phase was washed with water and brine, dried with K₂CO₃ or MgSO₄, filtered and concentrated under vacuum. The crude product was purified by column chromatography and further purified by preparative RP-HPLC (H₂O/ MeCN with 0.1% TFA) to isolate the pure compounds 129, 130, 133 and 134 as the TFA-salts.

N-tert-Butyl-7-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (129) and N-tert-Butyl-5-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (130). According to the general procedure a microwave vial (2-5 mL) was charged with the crude mixture of boronic acids 127 and 128
(135 mg, 0.454 mmol), compound 118 (57.9 mg, 0.244 mmol), 3.5 mL of toluene, 0.5 mL of ethanol, Na₂CO₃ (2 M, 0.42 mL, 0.840 mmol) and Pd(PPh₃)₄ (12.1 mg, 10.5 μmol). The crude product was purified by column chromatography (CH₂Cl₂:MeOH 20:1), followed by separation of the products using preparative RP-HPLC to afford the pure compounds 129 and 130 in a total yield of 88% (88.4 mg, 0.22 mmol, 129, 40.9 mg, 0.10 mmol, mix, 22.7 mg, 0.06 mmol, 130, 18.7 mg, 0.05 mmol).

129: ¹H NMR (CDCl₃), δ, ppm: 8.98 (brs, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.42–7.40 (m, 3H), 7.36 (d, A, A', J = 8.3 Hz, 2H), 7.31 (d, J = 8.0 Hz, 1H), 7.214–7.207 (m, 1H), 5.39 (s, 2H), 3.80 (brs, 1H), 3.01 (t, J = 7.6 Hz, 2H), 2.52 (t, J = 7.6 Hz, 2H), 2.03 (qui, J = 7.6 Hz, 2H), 1.09 (s, 9H). ¹³C NMR (CDCl₃), δ, ppm: 149.5, 145.7, 139.82, 139.77, 135.7, 135.0, 132.4, 131.1, 128.0, 127.3, 123.9, 121.4, 121.0, 54.7, 53.0, 33.4, 32.7, 30.1, 25.1.

130: ¹H NMR (CDCl₃, a few drops of CD₃OD), δ, ppm: 8.98 (brs, 1H), 7.95 (s, 1H), 7.51 (m, 2H), 7.40 (s, 1H), 7.33 (m, 2H), 7.19 (s, 1H), 7.06 (s, 1H), 5.36 (s, 2H), 2.96 (m, 4H), 2.18–2.12 (m, 2H), 0.99 (s, 9H).

N-tert-Butyl-7-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (133) and N-tert-Butyl-5-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (134). According to the general procedure a microwave vial (2–5 mL) was charged with the crude mixture of boronic acids 127 and 128 (132 mg, 0.444 mmol), compound 119 (62.7 mg, 0.264 mmol), 3.5 mL of toluene, 0.5 mL of ethanol, Na₂CO₃ (2 M, 0.42 mL, 0.840 mmol) and Pd(PPh₃)₄ (12.2 mg, 10.6/μmol). The crude product was purified by column chromatography (CH₂Cl₂:MeOH 20:1). A separation using preparative reversed-phase gradient HPLC (ACN/water) followed by separation of the products using preparative RP-HPLC to afford the pure compounds 133 and 134 in a total yield of 87% (94.5 mg, 0.23 mmol, 133, 47.5 mg, 0.12 mmol, mix, 19.8 mg, 0.05 mmol, 134, 20.7 mg, 0.05 mmol). Compound 133 was observed as two rotamers at 24 °C in ¹H NMR, the spectrum recorded at 24 °C is reported here 133: ¹H NMR (CDCl₃), δ, ppm: 8.95 (brs, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.39–7.26 (m, 6H), 5.44–5.27 (m, 2H), 3.67 (s, 1H), 3.04–3.00 (m, 2H), 2.68–2.60 (m, 1H), 2.48–2.40 (m, 1H), 2.09–2.01 (m, 2H), 1.07 (s, 9H). ¹³C NMR (CDCl₃), δ, ppm: 149.6, 145.6, 139.9, 139.3, 135.6, 135.1, 132.7, 131.3, 130.5, 129.3, 128.3, 127.5, 124.0, 121.3, 121.2, 54.8, 53.2, 33.5, 32.7, 30.2, 25.2.

134: ¹H NMR (CDCl₃), δ, ppm: 9.00 (brs, 1H), 7.99 (s, 1H), 7.69–7.63 (m, 1H), 7.54–7.42 (m, 3H), 7.39–7.34 (m, 1H), 7.28–7.26 (m, 1H), 7.12 (s, 1H), 5.38 (s, 2H), 3.64 (s, 1H), 3.15–2.94 (m, 4H), 2.22–2.12 (m, 2H), 1.01 (s, 9H). ¹³C NMR (CDCl₃), δ, ppm: 149.2, 144.8, 141.5, 139.9, 137.2, 135.5, 131.3, 130.7, 129.3, 128.9, 128.7, 128.1, 124.6, 121.3, 121.1, 54.7, 53.3, 33.0, 32.7, 30.0, 25.5.
General procedure for compounds 131, 132, 135 and 136. Compounds 129, 130, 133 and 143 were dissolved in dry CH₂Cl₂ and cooled to 0 °C and reacted with an excess of 1.0 M BCl₃ in hexane fractions under nitrogen. The reaction was left at room temperature for 1 hour. The reaction mixture was then evaporated and co-evaporated several times with CHCl₃. The residue was dissolved in CH₂Cl₂ and water (3:1). Na₂CO₃ was added and the reaction mixture was cooled to 0 °C and n-butyl chloroformate was added and the reaction was left to reach room temperature over night. The reaction mixture was diluted with CHCl₃ organic and the phases were separated. The organic phase was washed with water and brine, dried over MgSO₄ and evaporated. The residue was purified on preparative RP-HPLC (H₂O/MeCN with 0.1% TFA) to give the pure products 131, 132, 135 and 136 as the TFA-salts.

**N-(Butoxycarbonyl)-7-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (131).** According to the general procedure compound 129 (33 mg, 80.6 μmol) was dissolved in dry CH₂Cl₂ (1.5 mL) and reacted with BCl₃ (0.35 mL, 350 μmol). The crude product was dissolved in CH₂Cl₂ (1.5 mL) and water (0.5 mL) and reacted with Na₂CO₃ (38.7 mg, 365 μmol) and n-butyl chloroformate (14.5 μL, 114 μmol) and n-butyl chloroformate (14.5 μL, 114 μmol). Compound 131 was isolated as a white solid in 35% yield (12.8 mg, 28.2 μmol). ¹H NMR (CDCl₃), δ, ppm: 8.81 (brs, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.41–7.37 (m, 2H), 7.32–7.24 (m, 5H), 5.34 (brs, 2H), 4.03 (t, J = 6.7 Hz, 2H), 3.03 (t, J = 7.5 Hz, 2H), 2.49 (t, J = 7.5 Hz, 2H), 2.04 (qui, J = 7.5 Hz, 2H), 1.54–1.56 (m, 2H), 1.27–1.18 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H). ¹³C NMR (CDCl₃), δ, ppm: 151.20, 151.18, 145.9, 139.1, 136.1, 136.0, 134.8, 132.2, 130.3, 130.0, 128.3, 123.9, 121.3, 121.1, 66.7, 53.0, 33.6, 32.5, 30.6, 25.1, 18.9, 13.7.

**N-(Butoxycarbonyl)-5-[4-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (132).** According to the general procedure compound 130 (10 mg, 24.4 μmol) was dissolved in dry CH₂Cl₂ (0.5 mL) and reacted with BCl₃ (0.10 mL, 100 μmol). The crude product was dissolved in CH₂Cl₂ (0.8 mL) and water (0.3 mL) and reacted with Na₂CO₃ (11.4 mg, 108 μmol) and n-butyl chloroformate (4.3 μL, 34.2 μmol). Compound 132 was isolated as a white solid in 13% yield (1.4 mg, 3.09 μmol). ¹H NMR (CDCl₃), δ, ppm: 8.99 (brs, 1H), 8.10 (s, 1H), 7.98–88 (m, 1H), 7.45–28 (m, 5H), 7.12 (s, 1H), 5.40 (s, 2H), 4.03 (t, J = 6.6 Hz, 2H), 3.08–2.95 (m, 4H), 2.22–2.13 (m, 2H), 1.53–1.44 (m, 2H), 1.28–1.15 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H).

**N-(Butoxycarbonyl)-7-[3-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (135).** According to the general procedure compound 133 (20 mg, 48.8 μmol) was dissolved in CH₂Cl₂ (0.8 mL) and reacted with BCl₃ (0.20 mL, 200 μmol). The crude product was dissolved in CH₂Cl₂ (0.8 mL) and water (0.3 mL) and reacted with Na₂CO₃ (23.9 mg, 225 μmol) and n-
butyl chloroformate (8 µL, 63.0 µmol). Compound 135 was isolated as a white solid in 10% yield (2.0 mg, 4.41 µmol). Compound 135 was observed as two rotamers at 24 °C in $^1$H NMR. Due to high temperature sensitivity no high temperature experiments were performed and the spectrum recorded at 24 °C is reported here. $^1$H NMR (CDCl$_3$), δ, ppm: 9.05 (brs, 1H), 8.04 (d, $J = 8.0$ Hz, 1H), 7.80–7.58 (m, 1H), 7.52–7.46 (m, 1H), 7.41–7.34 (m, 3H), 7.24–7.16 (m, 2H), 5.55–5.32 (m, 2H), 4.03 (t, $J = 6.5$ Hz, 2H), 3.07–3.00 (m, 2H), 2.64–2.56 (m, 2H), 2.50–2.42 (m, 2H), 2.10–2.00 (m, 2H), 1.52–1.45 (m, 2H), 1.28–1.17 (m, 2H), 0.86 (t, $J = 7.3$ Hz, 3H).

$N$-(Butoxycarbonyl)-5-[3-(imidazol-1-ylmethyl)phenyl]-indan-6-sulfonamide (136). According to the general procedure compound 134 (15 mg, 36.6 µmol) was dissolved in CH$_2$Cl$_2$ (0.8 mL) and reacted with BCl$_3$ (0.15 mL, 150 µmol). The crude product was dissolved in CH$_2$Cl$_2$ (0.8 mL) and water (0.3 mL) and reacted with Na$_2$CO$_3$ (17.8 mg, 168 µmol) and n-butyl chloroformate (6.5 µL, 51.2 µmol). Compound 136 was isolated as a white solid in 36% yield (5.9 mg, 13.0 µmol). $^1$H NMR (CDCl$_3$), δ, ppm: 8.93 (brs, 1H), 8.08 (s, 1H), 7.47–7.43 (m, 1H), 7.34–7.23 (m, 5H), 7.12 (s, 1H), 5.38 (brs, 2H), 3.99 (t, $J = 6.6$ Hz, 2H), 3.03 (t, $J = 7.7$ Hz, 2H), 2.98 (t, $J = 7.4$ Hz, 2H), 2.21–2.13 (m, 2H), 1.50–1.42 (m, 2H), 1.24–1.14 (m, 2H), 0.85 (t, $J = 7.4$ Hz, 3H). $^{13}$C NMR (CDCl$_3$), δ, ppm: 150.9, 150.8, 144.8, 140.5, 138.3, 136.1, 135.0, 131.9, 130.3, 130.1, 129.3, 128.14, 128.10, 126.7, 121.2, 120.8, 66.7, 53.1, 33.0, 32.6, 30.5, 25.4, 18.8, 13.7.
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