The antihypertensive activity of benzimidazoles and the binding of its derivatives to the AT_1receptor

Abstract

To examine the antihypertensive effects of benzimidazole derivatives and the effect of different substituents, on the benzimidazole core, on the antihypertensive effect this paper looks at the binding of inverse agonists and agonists to the AT_1 receptor. It also compares the affinity to the receptor, the inverse agonistic activity, and the antihypertensive effects of two clinically approved antihypertensive drugs azilsartan and candesartan. The effect of different substitutents is examined in the discussion, as well as the effect of the different functional groups of azilsartan and candesartan on the antihypertensive effect of the compounds.

Contents

1 Introduction

According to the World Health Organization the most common cause of death is coronary heart disease, which means heart failure due to high blood pressure (hypertension) over time. Hypertension over time may lead to heart failure and stroke. Because of this there has been done an enormous amount of research on pharmaceutical medicaments with antihypertensive effects which lowers blood pressure. Many of these drugs works by interfering with the Renin-Angiotensin System (RAS). RAS is a cascade of enzymatic reactions that converts the polypeptide agiotensinogen into the decapeptide angiotensin I (Ang I), which is then converted into the oligopeptide angiotensin II (Ang II) by the help of the angiotensin converting enzyme (ACE). Ang II then reacts with its receptor angiotensin receptor 1 (AT_1), which in turn mediates several effects that leads to hypertension. Because of this there has been developed several classes of drugs that interfere with RAS. One of these are Renin and ACE inhibitors which block the production of Ang II, and another is angiotensin receptor blockers (ARBs) which blocks the binding of Ang II to the AT_1 receptor. The ARBs have been shown to be more effective than the Renin and ACE inhibitors, as Ang II can be produced in other ways than just by ACE catalysed reactions.[1] In 1994, the first ARB was introduced.[2] This drug is called losartan. Losartan is a imidazole ring with a methoxy group, chlorine, *n*-Bu, and a biphenyl group with a tetrazol ring as substituents.[3] Losartan is a surmountable inverse agonist. Eventually it was discovered that compounds with a benzimidazole cores where effective ARBs. Benzimidazoles are a class of organic compounds whom share the fundamental structure of a six-membered benzene fused to five-membered imidazole moiety.[4] Molecules with a benzimidazole nucleus show a wide-ranging biological activities such as antiviral activity, antimicrobial, antihypertensive and more. There were three ARBs with a benzimidazole core available on the U.S. market as of 2011, azilsartan, candesartan, and telmisartan.[5]All three of them are insurmountable inverse agonists Their structure is shown in 1.1 This paper will examine the biological activity of benzimidazol derivatives focusing on its antihypertensive effects and the effects different substituents have on the antihypertensive effects. It will also examine the binding between inverse agonists with benzimidazole cores and the AT_1. Two strong inverse agonists in azilsartan and candesartan will also be compared based on their binding to the AT_1 receptor, their affinity tothe receptor, and their antihypertensive and inverse agonistic activity.

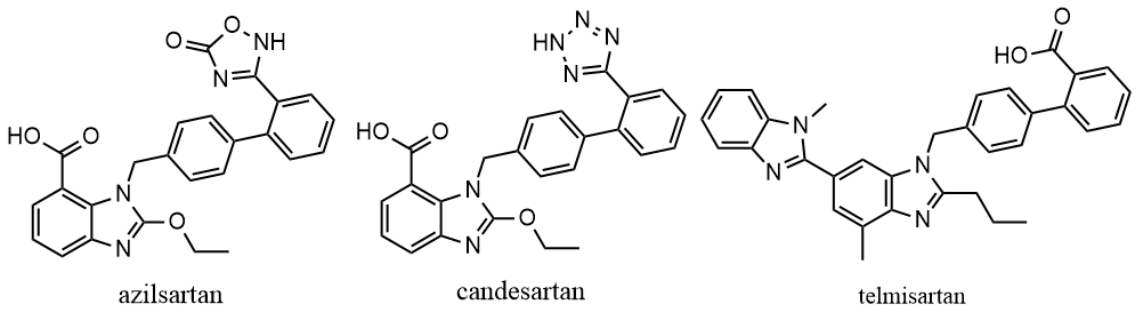

Figure 1.1: Structures of azilsartan, candesartan, and telmisartan

2 Theory

2.1 The Renin Angiotensin System (RAS)

The Renin Angiotensin System (RAS) is a cascade of enzymatic reactions where the polypeptide angiotensinogen is cleaved by renin, a protein, which results in the production of the decapeptide angiotensin I (Ang I). Ang I then reacts with angiotensin converting enzyme (ACE) and gets converted into the oligopeptide angiotensin II (Ang II). Ang II may then act on angiotensin receptor 1 (AT_1) and mediate vascoconstriction, Na^+ and aldosterone release which in turn leads to an increase in blood pressure.[1] Although the production of Ang II is mainly a results of Ang I reacting with ACE, Ang I may also be enzymatically converted to Ang II by chymase.[6] Because of this the blocking of AT_1 is believed to be the most effective way of causing antihypertensive effects.

2.2 Angiotensin receptor 1

The angiotensin receptor 1 is a polypeptide made up of 359 amino acids.[7] AT_1 is a part of the G protein-coupled receptor (GCPR) family and is a seven-transmembrane receptor, meaning it has seven transmembrane helices. The AT_1 does exhibit some but very minimal constitutive activity. Constitutive activity means that the receptor can exhibit some activity even without the presence of it's ligand.[8] Allthough the AT_1 receptor has a low basal activity, its Lys111Gly mutant receptor has a high basal activity which makes it usefull in studies examining the inverse agonistic activity of compounds.[9] An antagonist will only hinder further activation by a ligand but not hinder the constitutive activity. Therefore the angiotensin II receptor blockers (ARBs) are inverse agonists since they deactivate the receptor completely. Studies done on the transition of GCPR from an inactive state to an active state indicates that the inactive AT_1 is in a constricted form.[10] Interaction with Ang II changes the conformation of the receptor exposing important binding sites for AngII and results in the receptor expanding as it becomes active.[3] When activated by Ang II effects that lead to an increase in blood pressure such as aldosterone release, contraction of vascular smooth muscle cells, and Na^+ release are mediated.[1]

2.3 Angiotensin II

The oligopeptide Ang II consists of eight amino acids whom are Asp^1, Arg^2, Val^3, Tyr^4, Ile^5, His^6, Pro^7, Phe^8.[11] The structure is shown in figure 2.1 Studies done on the relationship between structure and activity has shown that the C-terminal region of the peptide and Arg^2, Tyr^4, His^6 and Phe^8 are important for the biological activity of AngII.[12] It has been shown that Asp^1 does not contribute to the biological activity of Ang II as studies show no significant change in activity when Asp^1 was replaced with other amino acids or with the removal of Asp^1 alltogether.[10]
The two most important amino acids for activating the receptor is Phe^8 and Tyr^4.[13] The COOH group of Phe^8 interacts with the ϵ-amino group on the Lys^{199} of the receptor, and forms an ionic bridge which positions the Phe^8 of AngII such that it can interact in an optimal way with the receptors His^{256}.[14] These interactions changes the conformation of the receptor which contributes heavily in giving the receptor its agonistic activity. The interactions between Phe^8 and Lys^{199} is also important for positioning Phe^8 over Pro^7 and stabilizng the bond between the two amino acids. This positioning results in an important conformational change of Ang II that is essential for the affinity for the receptor.[11]
The next interaction happens between Tyr^4 and Asn^{111}. In the receptors inactive state there exists an intramolecular hydrogen bond between Asn^{111} and Asn^{295} and/or Tyr^{292}.[15][16]. This intramolecular hydrogen bond is believed to be important for the receptors inactive state.[17] Tyr^4 binding to Asn^{111} breaks this intramolecular hydrogen bond and changes the conformation of the receptor so that it changes into its active state.

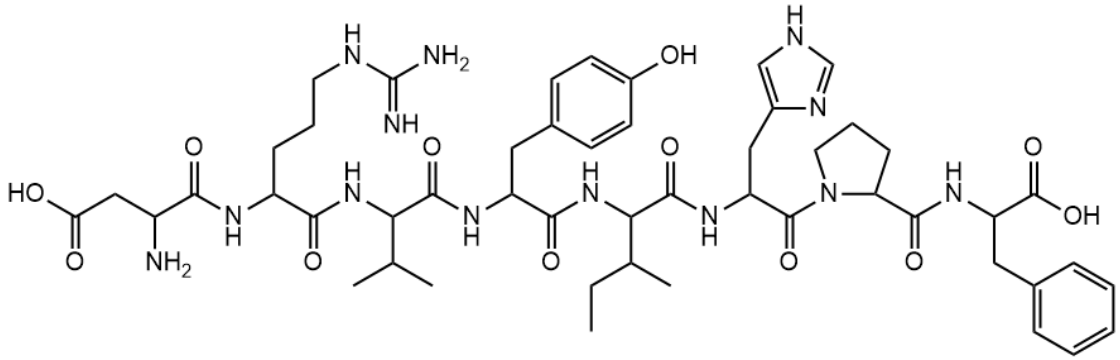

Figure 2.1: Structure of the oligopeptide angiotensin II

2.4 Inverse agonists binding to the AT_1 receptor

While peptide agonists and inverse agonists bind to the extracellular domain and the transmembrane domain of the receptor, non-peptide inverse agonists has been shown to interact with binding sites only in the receptors transmembrane domain. It has also been shown that many of the binding sites in the transmembrane domain is the same for agonists and inverse agonists.[8] Some non-peptide inverse agonists therefore work by interfering the agonist by binding in the same sites. This is known as competitive, surmountable inverse agonism and an example of this kind of inverse agonist is losartan. Insurmountable inverse agonists binds differently to the receptors transmembrane domain by binding more tightly and having a higher affinity for the receptor than the surmountable inverse agonists and therefore dissociating more slowly from the receptor.[7]Insurmountable inverse agonists may also bind to the receptor in a manner that hinders the conformational change needed for agonist binding.[18] Some examples of insurmountable inverse agonists are candesartan, telmisartan and azilsartan which all have a benzimidazole nucleus. For non-peptide inverse agonists to excert inverse agonistic activity they are dependent on the receptors Tyr^{113}, Lys^{119}, His^{256}, Gln^{257} and Asn^{295}.

2.5 Effects of different functional groups on antihypertensive effects

The functional groups on the benzimidazole nucleus plays a pivotal role in the effectivness of the compounds ability to provide an antihypertensive effect. Factors that influences a compounds antihypertensive effects are how well its functional groups fits in the AT_1 receptor pockets and the compounds affinity to the receptor, which in turn affects how slowly the compounds dissociates from the receptor and how long lasting a drug may be.

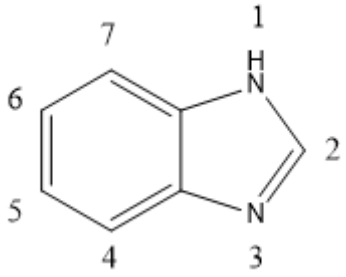

Figure 2.2: Benzimidazole and numbering of its atoms that can be substituted.

The N-3 of benzimidazole interacts with the receptor through H-bonding interactions. For this to be favorable the position 4 and N-3 must in general be unsubstituted.[1]. The position 5 can have a multitude of functional groups, but it has been established that it is favorable to have a

hydrophillic group that is not to big. These functional groups may be nitro, alkylcarboxamido, amino or alykl-arylsulfanoamido.[1] A study done by Shah et al. shows the effect of the size of the substituent at position 5. It took a benzimidazole with a *n*-Bu at position 2 and BPC at the substituted N and a carboxamido at the position 5, and compared different length of acylating reagents connected to the carboxamido. The reagents where methyl(a), ethyl(b), n-propyl(c), n-butyl(d), phenyl(e), 2-Cl-phenyl(f) and 4-Cl-phenyl(g). It also compared these compounds to losartan, candesartan, and a benzimidazole with a amino group at the position 5 and BPC at the substituted N and a *n*-Bu at position 2 (compound 1). All structures ar shown in figure 2.3. The in vitro antagonistic activity showed compounds a and b to be more potent than compounds c-g. Compound a was found to be more potent than compound b. Compound a exhibited greater activity than losartan and compound 1, but equivalent activity to candesartan. Compound b exhibited equivalent activity to compound 1, and more activity than losartan. Compounds c-g, with the most bulky residues, showed significantly less activity than the other compounds. Antihypertensive effect was also meassured, by measuring the decrease in mean arterial blood pressure (MABP), in vivo where it was found to correspond to the antagonistic activity of compounds a-g in vitro except for a and b, where b was found to have a greater antihypertensive effect than a. Compounds a and b exhibited greater antihypertensive effects than both losartan and candesartan. Compound a exhibited equivalent activity to that of compound 1.[19]

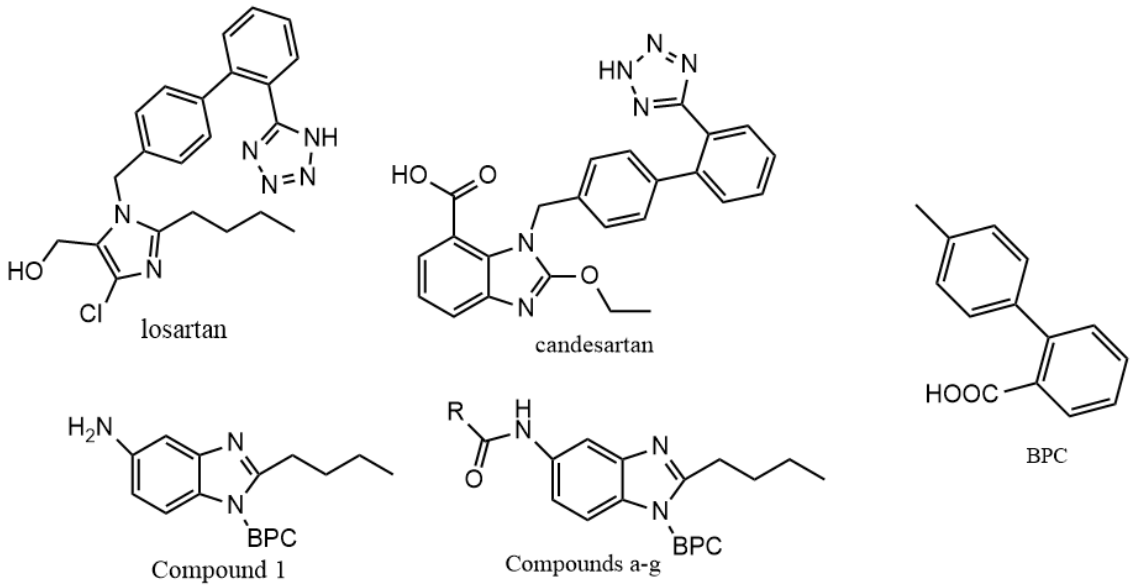

Figure 2.3: Structures of losartan, candesartan, compound 1, BPC and compounds a-g where R is methyl for compound a, ethyl for compound b, *n*-propyl for compound c, *n*-Bu for compound D, phenyl for compound e, 2-Cl-phenyl for compound f, and 4-Cl-phenyl for compound g

It has been found that the substituted N is reserved for an acidic biphenyl moiety. This enables the biphenyl part of the substituent to interact with the receptors lipophillic pocket through van der Waal interactons and for the acidic part to interact with the receptor through H-bonding.[20]

The most effective functional groups on the position 2 of the benzimidazole seems to be a short alkoxy chain, or a branched or linear propyl or butyl group.[1] Strong inverse agonists like telmisartan, azilsartan and candesartan shows this, with azilsartan and candesartan having an etoxy group at position 2, and telmisartan having a propyl group at position 2 of it's central benzimidazole.

A study done by W. Zhu et al. looked at the effect of different functional groups on the position 2 of 5-nitro benzimidazole derivatives. The study compared six different compounds numbered from 1 to 6, and losartan on their affinity to the AT_1 receptor and their in vivo antyhypertensive activity in spontaneously hypertensive rats (SHR). Compounds 1-6 are shown in 2.4 with R being et in compund 1, *n*-pr in compound 2, *n*-Bu in compound 3, *n*-Amyl in compound 4, *n*-pr in compound 5, and *n*-Bu in compound 6. Losartan is has a *n*-Bu substituent at its imidazole ring. The study showed that compounds 3 and 5 had the highest affinity to the AT_1 receptor while compound 4 exhibited significantly lower affinity to the AT_1 receptor. The in vivo antihypertensive activity was examined by looking at the effects of compounds 1-6 and losartan on the mean blood pressure (MBP) of SHR. As expected compound 4, which exhibited the lowest affinity to the AT_1 receptor, failed to significantly decrease the MBP compared to the control group who were not given anything to decrease the MBP. Compound 2 managed to lower the MBP but not enough to be deemed usefull. Losartan and compounds 1, 5, and 6 lowered the MBP by quite similar amounts. The duration of antihypertensive effects of losartan and compound 1 were very similar, while the duration of antihypertensive effects of compounds 5 and 6 were found to be lower than compound 1 and losartan. Compound 3 lowered the MBP the most of any compound and exhibited better antihypertensive effects after 24 hours than losartan. Therefore in this study the most effective substitution on the position 2 was shown to be *n*-Bu.[21]

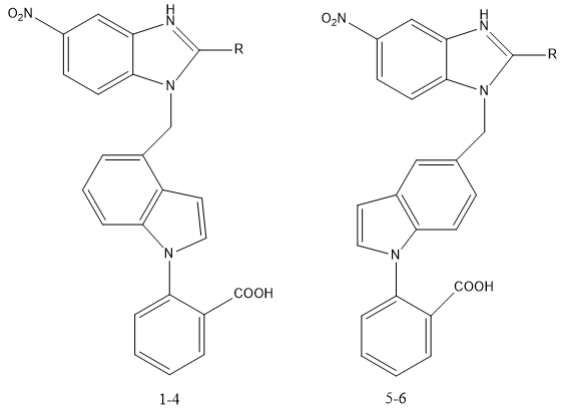

Figure 2.4: Illustration of compounds 1-6 in study done by W. Zhu et al. R being et in compund 1, *n*-pr in compound 2, *n*-Bu in compound 3, *n*-Amyl in compound 4, *n*-pr in compound 5, and *n*-Bu in compound 6

It is suggested that if the postition 6 on the benzimidazole core was to be substituted it would be best for the functional group to be a short lipophillic/electronic group.[1] The functional group on the position 7 should be a carboxylate group, as this group binds well to the AT_1 receptors Lys^{199}. Azilsartan and candesartan both have a carboxylate group on the position 7 of the benzimidazole core.

2.6 Candesartan

Candesartan is a potent ARB that lowers the blood pressure of hypertensive patients. Candesartan is defined as an insurmountable inverse agonist, as it surpresses the basal activity of the AT_1 receptor. The structure of candesartan is shown in figure 2.5. At the substituted N of candesartans benzimidazole core there i a biphenyl group with a tetrazole ring at the end of it. It has a carboxylate group on the postition 7 of the benzimidazole core, and an etoxy group at the second position. These functional groups are important in the way that candesaran binds to the At_1 receptor. In a study done by Miura et al. the binding affinity of candesartan to the AT_1 receptor and some of its mutants. Candesrtan showed a reduction, of more than tenfold, in its affinity for the Tyr114Ala, Lys199Ala, Gln257Ala, and Asn297Ala

receptor mutants. The tetrazol ring forms a H-bond with the Gln^{257} of the At_1 receptor, with Miura et al. reporting that the bond length is 3.3Å.[9] The same study also reports that the biphenyl group of candesartan interact with Tyr^{113} of the AT_1 receptor by van der Waals interactions with the reported bond length being 4.0Å. Its carboxylate group is believed to bind to the AT_1 receptors Lys^{199} by forming a salt bridge.[18] Candesartan also binds to the AT_1 receptors lipophillic pocket delimited by Ser^{105}, Trp^{253}, His^{256}, Ile^{288}, and Tyr^{292} which is located between the transmembrane helices 3 and 6.[3]

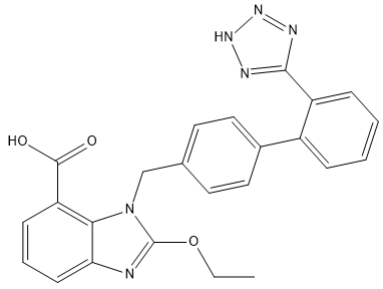

Figure 2.5: Structure of the ARB candesartan

2.7 Azilsartan

Another strong ARB is azilsartan. It is a strong inverse agonist that binds tightly to and dissociates slowly from the AT_1 receptor.[3] Azilsartan is very similar to candesartan in its structure, with the only difference being that azilsartan has a oxadiazole ring in place of candesartans tetrazol ring. The structure of azilsartan is shown in figure 2.6. In the same study by Miura et al., referenced in the subsection about candesartan, azilsartans affinity for several receptor mutants was examined with Tyr113Ala, Lys199Ala, Gln257Ala, and Asn295Ala receptor mutants reducing the affinity of azilsartan by more than 10-fold. It was suggested that the oxadiazole ring formed a H-bond with the AT_1 receptors Gln^{257}, with the bond length reported to be 2.6Å. Tyr^{113} of the AT_1 receptor interacts with the biphenyl group of azilsartan by van der Walls interaction, with the reported bond length being 3.4Å. The carboxylate group formes a salt bridge with Lys^{119}. The same study also showed that azilsartan-7H, which is azilsartan with a hydrogen at the position 7 of the benzimidazole core instead of a carboxylate group, had a much lower affinity for the AT_1 receptor than both azilsartan and candesartan. Azilsartan-7H also failed to show inverse agonistic effect on the AT_1 receptor and instead showed neutral antagonistic effect.[9]

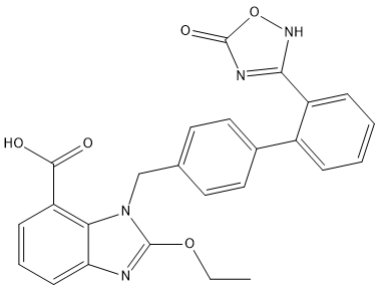

Figure 2.6: Structure of the ARB azilsartan

3 Discussion

As previously stated azilsartan and candesartan have very simillar structures. The only difference being that candesartan has a tetrazol ring at the end of the biphenyl group, while azilsartan has a oxadiazole ring at the same postition. Both candesartan and azilsartan showed a greater than 10-fold reduction in their affinity to the Tyr113Ala, Lys199ALa, Gln257Ala, and Asn295Ala receptor mutants. This indicates that it is critical that candesrtan and azilsartan interact with the AT_1 receptors Tyr^{113}, Lys^{199}, Gln^{257}, and Asn^{295} Both are insurmountable inverse agonists , meaning they both lower the basal activity of the AT_1 receptor. Miura et al. examined the inverse agonistic activity of candesartan and azilsartan. The basal activity of the receptor was too low to estimate the difference in inverse agonistic activity of candesartan and azilsartan. Because of this the Lys111Gly, which has a higher basal activity, was used to examine the difference in the inverse agonistic activity of the compounds. Azilsartan lowered the basal activity more than candesartan, meaning azilsartan exhibited stronger inverse agonistic activity than candesartan.[9] Candesartan and azilsartan interacts with the AT_1 receptor similarly to eachother. Comparing the H-bond between Gln^{257} of the receptor and the tetrazol ring of candesartan, and between Gln^{257} and the oxadiazole ring of azilsartan we see that the reported bond length is 3.3Å and 2.6Å respectively. The shorter bond length of the oxadiazole ring with Gln^{257} compared to the bond length of the tetrazol ring with Gln^{257} may indicate that the H-bond between the oxadiazole ring and Gln^{257} is stronger than that between the tetrazole ring and Gln^{257}. The bond length between the van der Waals interaction between the biphenyl group of the compounds and Tyr^{113} was also shorter for azilsartan than for candesartan, indicating that this bond may also be stronger for azilsartan. The low receptor affinity of azilsartan-7H indicates that the interaction between the carboxylate group of azilsartan and candesartan, and the receptors Lys^{199} is important for binding between the compounds and the receptor. The failure of azilsartan-7H to exhibit inverse agonistic activity indicates that the salt bridge between the carboxylate group and Lys^{199} is important for inverse agonistic binding.[9] Rakugi et al. showed that the antihypertensive effect of azilsartan was higher than that of other ARBs. The antihypertenisve effects of azilsartan over 24 hours was more potent than that of candesartan. This may be due to the strong binding of azilsartan to the AT_1 receptor and the slow disociation from the receptor. Although the difference between the two compounds is minimal the replament of the tetrazol ring with a oxadiazole ring results in better antihypertensive effects and greater affinity to the AT_1 receptor.

In the study done by W. Zhu et al., and referenced in the subsubsection 2.2.5, different substituents on the position 2 of 5-nitro benzimidazole derivatives was compared with each other and losartan based on their affinity to the AT_1 receptor and their antihypertensive effects. Compound 4, substituted with *n*-Amyl, exhibited the by far worst affinity to the receptor. Compounds 3 and 5, which were substituted with *n*-Bu and *n*-pr respectively at the position 2, exhibited the highest affinity to the receptor. Suggesting that compounds 3 and 5 would exhibit best in vivo antihypertensive effects. Examining the effects of compound 1-6 and losartan on the MBP og SHR, compound 3 exhibited better antihypertensive effects than the other compounds, while compound 5 exhibited simillar antihypertensive effects to that of compounds 1, 6, and losartan. This suggests that length of the *n*-Bu chain was more suitable for interactions with the receptor than the length of the *n*-pr chain. Since both compounds 3 and 6 were substituted with *n*-Bu at the position 2, but compound 3 exhibited significantly better antihypertensive effects it may suggest that the 1,4-disubstituted indole group has better activity than the 1,5-disubstituted indole group. The antihypertensice effects of compound 3 was found to be more potent and last longer than that of losartan, lasting for more than 24 hours. The study suggests abinding profile in which the alkyl chain on the position 2 interacts with a lipophillic pocket called L1 on the receptor. It is suggested that the length of the *n*-Bu lets it interact quicker and bind more tightly to the receptors lipophillic pocket L1. This interaction between the substituent at the position 2 and the receptors L1 pocket may explain why compound 4 with *n*-Amyl at the position 2 exhibited significantly lower binding affinity to the receptor and antihypertensive effects than the other compounds.[21] There may be two

main factors contributing to this. The first being the length og the *n*-Amyl being to long for it to interact well with the receptor, and the second being that its OH- group at the end does not interact well with the lipophillic pocket of the receptor. Azilsartans and candesartans ethoxy group at the postition 2 of its benzimidazole nucleus supports the suggestion that the substituent on position 2 should be a alkyl or alkoxy of an appropriate length.

The study conducted by Shah et al. found that its compound a, shown in figure 2.3, in vitro exhibited better antagonistic activity than losartan and its compound 1, and equivalent activity to that of candesartan. Compound b exhibited greater activity than losartan, and equivalent activity to that of compound 1. Compounds cg exhibited significantly less activity than the other compounds. This may suggest that the bulky alkyl or aryl groups of compounds c-g may interfere with the interactions between the NH-group and the studies proposed lipophillic pocket L4 delimited by Leu^{112}, Try^{253}, His^{256}, and Tyr^{292}. This suggests that a small lipophillic group like methyl or ethyl may be favoured to not interfere the interactions between the NH-group and the receptors L4 pocket. Compound b having a greater antihypertensive effect in vivo than compound a may suggessts that the ethyl group makes compound b favorable for optimal partitioning in body fluids which in turn leads to better in vitro activity.[19] Final remark: The in vitro activity was described as antigonistic in the study. As both losartan and candesartan are inverse agonists and not antagonist they show inverse agonistic activity, not antagonistic. To stay true to the study the activity of losartan and candesartan was desribed as antagonistic instead of inverse agonistic.

As seen in the studies referenced in this paper small changes of the functional groups on the benzimidazole core may significantly alter the compounds affinity to the AT_1 receptor and its inverse agonistic effects. There is still a lot of research to do on the effect of different substituents on the benzimidazole core. Since the interaction between the carboxylate group on the benzimidazole core and the AT_1 receptors Lys^{199} further research of different substituents on the position 7 may not be the most urgent. The reason interaction with Lys^{199} is important for inverse agonists may be because of the importance of Lys^{199} for Ang II. A critical interaction for Ang II to bind to the AT_1 receptor and exert agonistic activity is the interaction between Phe^8 of Ang II and the AT_1 receptors Lys^{199}. This interaction triggers a conformational change in the receptor which is important for its agonistic activity. It also triggers a conformational change in ANG II which is essential for the affinity of Ang II towards the receptor. When an inverse agonist binds to Lys^{199} it inhibits som of Ang II most critical interactions with the receptor. In a study done by Miura et al. [Sar^1]Ang II exhibited significantly less agonistic activity with the Asn111Gly/Lys199Gln receptor mutant than with regular AT_1 receptor.[9]
When it comes to the substituent on the substituted N in the benzimidazole core, it seems that a biphenyl moiety results in the best affinity for the AT_1 receptor and inverse agonistic activity. It seems like thus far the most effective group on the biphenyl group is a oxadiazole ring, but this may be researched further.
As for the postition 5 it might be usefull to research this position more. In the study done by Shah et al. compounds a and b of the study exhibited greater antihypertensive effects than losartan, and more importantly candesartan. This may indicate that the position 5 may be of importance for the antihypertensive activity og inverse agonists. Neither candesartan or azilsartan has a substituent on the postiion 5 of its benzimidazole core. N-3 and position 4 does not need much more research as it should not have any substituents. All in all there is still a lot of reesearch to be done on different substituents on the different positions of the benzimidazole core which will most likely lead to even more potent and long lasting antihypertensive drugs.

4 Conclusions

Azilsartan was shown by Miura et al. to have greater affinity to the AT_1 receptor than candesartan. The shorter bond length between azilsartans oxadiazole ring and the AT_1 receptors Gln^{257} compared to that between candesartans tetrazol ring and the receptor Gln^{257} may indicate that it binds tighter to the receptor than candesartan does. The shorter bond length for azilsartan rather than candesartan is seen in the interaction between the biphenyl groups of azilsartan and candesartan, and the receptors Tyr^{113}. This is likely to, among other factors, contribute to the greater antihypertensive effect of azilsartan. [9]

It has been found that the optimal substituents for the different positions of the benzimidazole core is as follows: A biphenyl moiety with an acidic group attached at the end. This is important for van der Waals interactions and H-bonding between the ligand and the receptor. The second position should have a short, C3-C4, linear or branched aryl or alkyl chain. The N-3 and position 4 should remain unsubsstituted in order to allow the N-3 to interact with the receptor through H-bonding. According to the results in the study done by Shah et al. the substituent on the position 5 should be a lipohillic group capable of forming H-bonds with the receptor. This group should not be too buky so that it avoids unfavorable steric interaction with the receptors L4 pocket. [19] It has been suggested that the best substituent on the postition 6 is a short lipophillic/electronic group. [1] The position 7 should be a carboxylate group as it has shown its importance in binding to the receptors Lys^{199} in turn increasing the inverse agonists affinity to the receptor and not letting Ang II interact with Lys^{199} which is critical for its agonistic activity.

All in all there is still a lot of reesearch to be done on different substituents on the different positions of the benzimidazole core which will most likely lead to even more potent and long lasting antihypertensive drugs.

Trondheim, May 3, 2021

Matias Aracena-Solem

References

[1] Y. Bansal and O. Silakari, "The therapeutic journey of benzimidazoles: A review," *Bioorganic Medicinal Chemistry*, vol. 20, no. 21, pp. 6208–6236, 2012.

[2] A. Gradman, "At 1-receptor blockers: differences that matter," *Journal of human hypertension*, vol. 16, no. 3, pp. S9–S16, 2002.

[3] P. Balakumar and G. Jagadeesh, "Structural determinants for binding, activation, and functional selectivity of the angiotensin at1 receptor," *Journal of molecular endocrinology*, vol. 53, no. 2, pp. R71–R92, 2014.

[4] K. Kamanna, "Synthesis and pharmacological profile of benzimidazoles," in *Chemistry and Applications of Benzimidazole and its Derivatives*, ch. 4, IntechOpen, 2019.

[5] K. Zaiken and J. W. Cheng, "Azilsartan medoxomil: a new angiotensin receptor blocker," *Clinical therapeutics*, vol. 33, no. 11, pp. 1577–1589, 2011.

[6] F. Fyhrquist and O. Saijonmaa, "Renin-angiotensin system revisited," *Journal of internal medicine*, vol. 264, no. 3, pp. 224–236, 2008.

[7] M. De Gasparo, K. Catt, T. Inagami, J. Wright, and T. Unger, "International union of pharmacology. xxiii. the angiotensin ii receptors," *Pharmacological reviews*, vol. 52, no. 3, pp. 415–472, 2000.

[8] S.-i. Miura, Y. Kiya, H. Hanzawa, N. Nakao, M. Fujino, S. Imaizumi, Y. Matsuo, H. Yanagisawa, H. Koike, I. Komuro, *et al.*, "Small molecules with similar structures exhibit agonist, neutral antagonist or inverse agonist activity toward angiotensin ii type 1 receptor," *PLoS One*, vol. 7, no. 6, p. e37974, 2012.

[9] S.-i. Miura, A. Okabe, Y. Matsuo, S. S. Karnik, and K. Saku, "Unique binding behavior of the recently approved angiotensin ii receptor blocker azilsartan compared with that of candesartan," *Hypertension Research*, vol. 36, no. 2, pp. 134–139, 2013.

[10] L. Oliveira, C. M. Costa-Neto, C. R. Nakaie, S. Schreier, S. I. Shimuta, and A. C. Paiva, "The angiotensin ii at1 receptor structure-activity correlations in the light of rhodopsin structure," *Physiological reviews*, vol. 87, no. 2, pp. 565–592, 2007.

[11] G. V. Nikiforovich and G. R. Marshall, "3d model for tm region of the at-1 receptor in complex with angiotensin ii independently validated by site-directed mutagenesis data," *Biochemical and biophysical research communications*, vol. 286, no. 5, pp. 1204–1211, 2001.

[12] B. C. Wilkes, L. Masaro, P. W. Schiller, and K. A. Carpenter, "Angiotensin ii vs its type i antagonists: conformational requirements for receptor binding assessed from nmr spectroscopic and receptor docking experiments," *Journal of medicinal chemistry*, vol. 45, no. 20, pp. 4410–4418, 2002.

[13] S.-i. Miura, Y.-H. Feng, A. Husain, and S. S. Karnik, "Role of aromaticity of agonist switches of angiotensin ii in the activation of the at1 receptor," *Journal of Biological Chemistry*, vol. 274, no. 11, pp. 7103–7110, 1999.

[14] K. Noda, Y. Saad, and S. S. Karnik, "Interaction of phe8 of angiotensin ii with lys199 and his256 of at1 receptor in agonist activation," *Journal of Biological Chemistry*, vol. 270, no. 48, pp. 28511–28514, 1995.

[15] T. Groblewski, B. Maigret, R. Larguier, C. Lombard, J.-C. Bonnafous, and J. Marie, "Mutation of asn111 in the third transmembrane domain of the at1a angiotensin ii receptor induces its constitutive activation," *Journal of Biological Chemistry*, vol. 272, no. 3, pp. 1822–1826, 1997.

[16] A. J. Balmforth, A. J. Lee, P. Warburton, D. Donnelly, and S. G. Ball, "The conformational change responsible for at1 receptor activation is dependent upon two juxtaposed

asparagine residues on transmembrane helices iii and vii," *Journal of Biological Chemistry*, vol. 272, no. 7, pp. 4245–4251, 1997.

[17] L. Hunyady, H. Ji, G. Jagadeesh, M. Zhang, Z. Gaborik, B. Mihalik, and K. Catt, "Dependence of at1 angiotensin receptor function on adjacent asparagine residues in the seventh transmembrane helix," *Molecular Pharmacology*, vol. 54, pp. 427–434, 1998.

[18] T. Tuccinardi, V. Calderone, S. Rapposelli, and A. Martinelli, "Proposal of a new binding orientation for non-peptide at1 antagonists: Homology modeling, docking and three-dimensional quantitative structure- activity relationship analysis," *Journal of medicinal chemistry*, vol. 49, no. 14, pp. 4305–4316, 2006.

[19] D. I. Shah, M. Sharma, Y. Bansal, G. Bansal, and M. Singh, "Angiotensin ii – at1 receptor antagonists: Design, synthesis and evaluation of substituted carboxamido benzimidazole derivatives," *European Journal of Medicinal Chemistry*, vol. 43, no. 9, pp. 1808–1812, 2008.

[20] A. Bali, Y. Bansal, M. Sugumaran, J. S. Saggu, P. Balakumar, G. Kaur, G. Bansal, A. Sharma, and M. Singh, "Design, synthesis, and evaluation of novelly substituted benzimidazole compounds as angiotensin ii receptor antagonists," *Bioorganic Medicinal Chemistry Letters*, vol. 15, no. 17, pp. 3962–3965, 2005.

[21] W. Zhu, Y. Da, D. Wu, H. Zheng, L. Zhu, L. Wang, Y. Yan, and Z. Chen, "Design, synthesis and biological evaluation of new 5-nitro benzimidazole derivatives as at1 antagonists with anti-hypertension activities," *Bioorganic Medicinal Chemistry*, vol. 22, no. 7, pp. 2294–2302, 2014.