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Interconversion of Functional Activity by Minor Structural Alterations in Nonpeptide AT_2 Receptor Ligands

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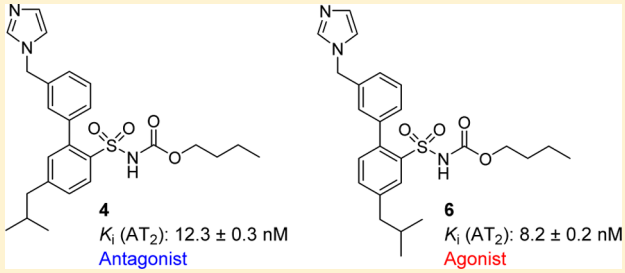
Supporting Information

ABSTRACT: Migration of the methylene imidazole side chain in the first reported selective drug-like AT_2 receptor agonist C21/M024 (**1**) delivered the AT_2 receptor antagonist C38/M132 (**2**). We now report that the AT_2 receptor antagonist compound **4**, a biphenyl derivative that is structurally related to **2**, is transformed to the agonist **6** by migration of the isobutyl group. The importance of the relative position of the methylene imidazole and the isobutyl substituent is highlighted herein.



KEYWORDS: *AT_2 receptor, nonpeptide ligands, agonist, antagonist, C21/M024, C38/M132*

The sartans (angiotensin II receptor blockers, ABRs) have been on the market as antihypertensives for more than two decades and act as selective antagonists at the angiotensin II AT_1 receptor. More recently, the angiotensin AT_2 receptor has emerged as a new target for drug intervention.[1−3] In 2004, the first selective and drug-like AT_2 receptor agonist C21/M024 (**1**) was disclosed,[4] and thereafter, a series of structural analogues with agonistic properties have been reported.[5,6] The activation of the AT_2 receptor is known to render a large number of diverse biological responses.[7,8] For example, receptor activation stimulates neurite outgrowth in neuronal cells, which is one of the first steps in neuronal differentiation.[9]

In healthy adults the AT_2 receptor expression is low and mainly found in renal, cardiovascular, and brain tissues.[7,8] Notably, during certain pathological conditions, such as myocardial infarction, vascular injury, brain ischemia, renal failure, and cutaneous wounds, the AT_2 receptor expression is significantly up-regulated.[7,8]

Recently, several studies conducted in experimental disease models have revealed that the selective potent AT_2 receptor agonist **1** acts as an anti-inflammatory agent and exerts pronounced tissue protective effects in particular in cardiovascular and renal disease. Hence, the compound has had a large impact on the view of the AT_2 receptor as a drug target.[1,9−14] In addition, the AT_2 receptor is a promising drug target for antagonists. Thus, the AT_2 receptor antagonist EMA401 has demonstrated convincing efficacy data in patients suffering from neuropathic pain (postherpetic neuralgia) in a phase II study.[3]

We discovered that migration of the methylene imidazole substituent of **1** from the para to the meta substitution pattern delivered a selective AT_2 receptor antagonist, compound C38/M132 (**2**) (Figure 1).[15,16] Thus, a minor structural alteration resulted in the interconversion of a selective AT_2 receptor agonist to a selective AT_2 receptor antagonist.

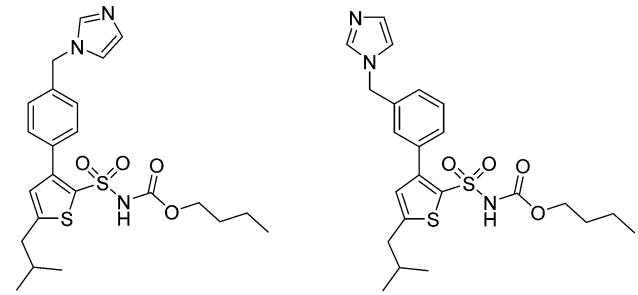


Figure 1. Structures of compounds **1** and **2**.

Herein, we report the synthesis and biological evaluation of four AT_2 receptor ligands, the biphenyl derivatives, and regioisomers, **3**, **4**, **5**, and **6** (Figure 2), and that the relative position of the imidazole and isobutyl substituent determines the functional outcome.

The nonselective Ang II receptor ligand **7** (L-162,782) acts as an AT_1 receptor agonist and **8** (L-162,389) acts as an AT_1

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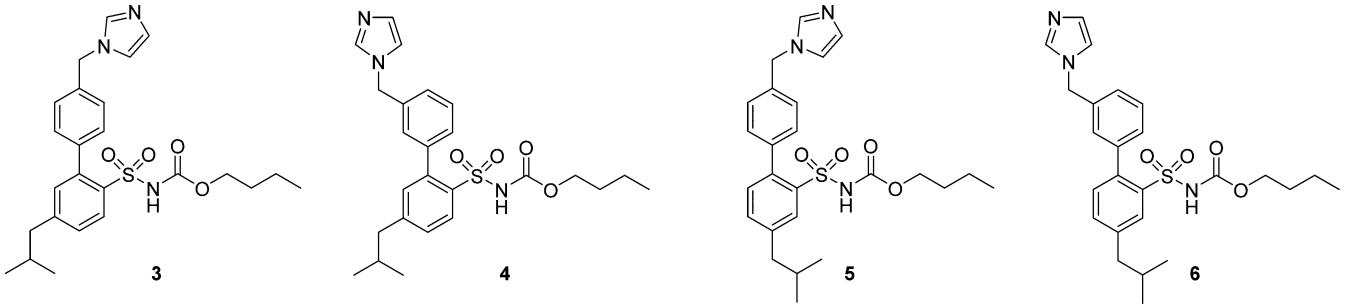

Figure 2. Structures of the regioisomers **3**, **4**, **5**, and **6**.

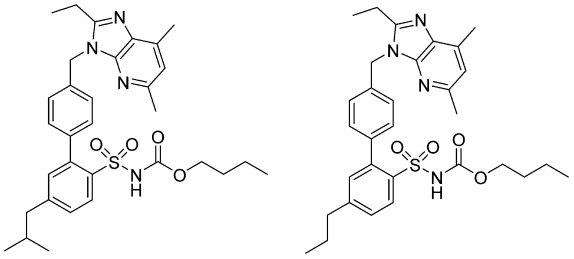

Figure 3. Structures of compounds **7** and **8**.

receptor antagonist (Figure 3).[17] Notably, these two structures differ only by a methyl group; the isobutyl group gives rise to agonism, while the *n*-propyl group renders antagonism.[17]

Since the only structural difference between the agonist **1** and the antagonist **2** can be found in the position of the methylene imidazole substituent, it can be hypothesized that the change in functionality could be attributed to a reduced ability to stabilize the activated receptor conformation due to a differently positioned imidazole group in **2**. However, as an alternative hypothesis the imidazole rings could have the same receptor interaction point, while the isobutyl groups are forced to be positioned differently and cause the difference in functionality (Figure 4A). If so, the topography and location of the lipophilic side chain of ligands binding to the AT_2 receptor is the primary determinant of functionality as was the case at the AT_1 receptor (cf. **7** and **8**).

In order to explore this alternative hypothesis and to identify a potential binding mode, a computational analysis was performed. Potential pharmacophore groups, including the imidazole rings, of **1** and **2** were superimposed using the pharmacophore modeling program DISCOtech[18] in Sybyl[19] (see Supporting Information for details). In order to reduce the number of conformations, model structures with methyl instead of butyl substituents on the sulfonylcarbamate group were used in the modeling. From the suggested binding mode model identifide, shown in Figure 4A, the isobutyl groups are oriented differently in the two thiophene compounds, which could be of importance for their ability to induce either the agonistic or antagonistic response. On the basis of this model, we hypothesized that it should be possible to convert the meta-substituted antagonist **2** to an agonist if the isobutyl chain could adopt a similar orientation as in the para-substituted agonist **1**, illustrated in Figure 4A. To investigate this hypothesis we performed further modeling where we replaced the thiophene

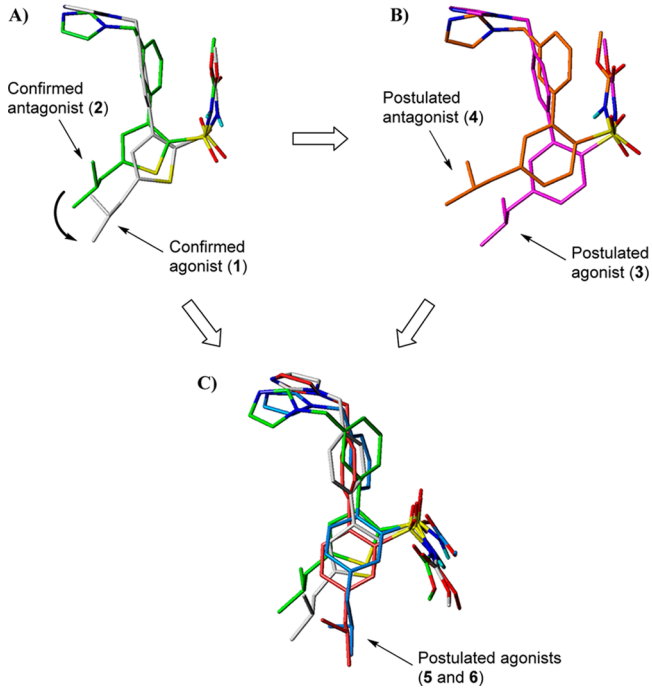

Figure 4. (A) Possible binding mode of two model compounds corresponding to structures **1** (white) and **2** (green). (B) Corresponding hypothesized binding mode for the two model compounds corresponding to the biphenyl analogues, **3** (purple) and **4** (orange), and postulated functions at the AT_2 receptor. (C) Comparison of the suggested binding modes of **1** and **2** with the analogues of **3** and **4**, where the isobutyl have been moved to the meta-position, the two model compounds corresponding to compounds **5** (red) and **6** (blue), respectively.

ring with a phenyl group to obtain biphenyl analogues allowing better assessment of the impact of relative positions of the isobutyl side chain (i.e., **3** and **4**, see Figure 2). Accordingly, superimposition of **3** and **4** produced a model, depicted in Figure 4B, which was very similar to that shown in Figure 4A. The corresponding analogues with an isobutyl group meta to the sulfonylcarbamate, compounds **5** and **6** (Figure 2), were also modeled and compared with the suggested binding modes of **1** and **2** as seen in Figure 4C. Although the position of the isobutyl group in **6** did not superimpose optimally to the corresponding group in **1**, it was clearly closer to this binding mode than that of **2**, where the imidazole and the sulfonylcarbamate also could reach the same interactions (Figure 4C). According to the model in Figure 4C, compound **5**, although having a meta-methylene imidazole substitution, could adopt a conformation that allows a similar binding mode as **6** and was therefore also hypothesized to act as an agonist. In order to

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explore this binding mode hypothesis we set out to synthesize regioisomers **3**–**6** and determine their AT_2 receptor binding affinity and, in particular, their functional activity at the AT_2 receptor.

The synthesis of **3** has been previously described.[5] The synthesis of compounds **4**–**6** are outlined in Scheme 1 and started with the benzenesulfonamides **9** and **10**, prepared as previously described by Wallinder et al.[20] Compound **13** and **14** were prepared by *N*-alkylation of imidazole by 3- and 4-bromobenzyl bromide in DMF. The crude boronic acids (**11** and **12**) were coupled with compounds **13** and **14** under Suzuki coupling conditions with $Pd(PPh_3)_4$ as catalyst and Na_2CO_3 as base using microwave irradiation (150 °C, 5 min) to form compounds **15**–**17** in good yields (75–87%). Compounds **4**–**6** were afforded after deprotection of the sulfonamide with BCl_3 and subsequent acylation with *n*-butyl chloroformate in DCM, water, and Na_2CO_3 to yield the final products in 27–51% yield, after purification by preparative HPLC.

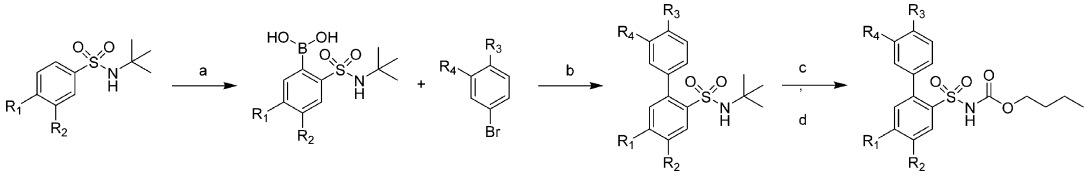
Compounds **3**–**6** were evaluated for affinity in a radioligand-binding assay by displacement of [^{125}I]Ang II from AT_2 receptors in pig uterus membranes as described previously by Nielsen et al.[21] The natural substrate Ang II and the selective AT_1 receptor antagonist losartan were used as reference substances.[22] The results of the binding study are presented in Table 1. Only AT_2 receptor affinity was evaluated since no AT_1 receptor binding have been seen for these methylene imidazoles even upon modifications of the lower part of the structures. This is also verified by compound **3**, which previously has been tested for AT_1 receptor affinity (K_i > 10 000 nM).[5]

Table 1. Biological Activities at the AT_2 Receptor

compd	K_i ± SD (nM)	function (10 nM)
3	0.7 ± 0.1	agoinst
4	12.3 ± 0.4	antagonist
5	10.1 ± 0.4	agonist
6	8.2 ± 0.2	agonist

All compounds were evaluated for functionality in a neurite outgrowth assay with NG108-15 cells. The NG108-15 cells express mainly the AT_2 receptor in their undifferentiated state, and a three-day treatment with Ang II or the selective peptidic AT_2 receptor agonist CGP-42112 (N_α-nicotinoyl-Tyr-(N_α-Cbz-Arg)-Lys-His-Pro-Ile)[23,24] induce neurite outgrowth.[16,25] The signaling pathways involve a sustained increase in Rap1/B-Raf/p42/p44^{mapk} activity and activation of the nitric oxide/guanylyl cyclase/cGMP pathway.[26,27] Cells were treated in the absence or presence of Ang II, **3**, **4**, **5**, and **6**. After 3 days of treatment, cells were examined under a phase-contrast microscope and micrographs were taken. To establish an adequate test concentration, the compounds were tested at various concentrations ranging from 0.1 to 100 nM. No evidence of cell death was observed. All compounds were tested at a concentration of 10 nM, compound **4** was also tested at 100 nM to verify that the lack of neurite outgrowth was not due to too low concentration (data not shown). Coincubation with the selective AT_2 receptor antagonist PD 123,319 reduced neurite outgrowth, verifying that the effect was mediated through the AT_2 receptor. Treatment with PD 123,319 alone did not alter the morphology compared to untreated cells (data not shown), as we have shown previously.[16] The antagonistic effect was verified through coincubation with Ang II resulting in reduced neurite outgrowth.

Scheme 1[a]



	R_1	R_2	R_3	R_4
9, 11		H	-	-
10, 12	H		-	-
13	-	-		H
14	-	-	H	
15, 4		H	H	
16, 5	H			H
17, 6	H		H	

[a]Reagents: (a) *n*-BuLi, THF, triisopropyl borate, HCl (aq); (b) $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O; (c) BCl_3, DCM; (d) *n*-butyl chloroformate, Na_2CO_3, DCM, H_2O.

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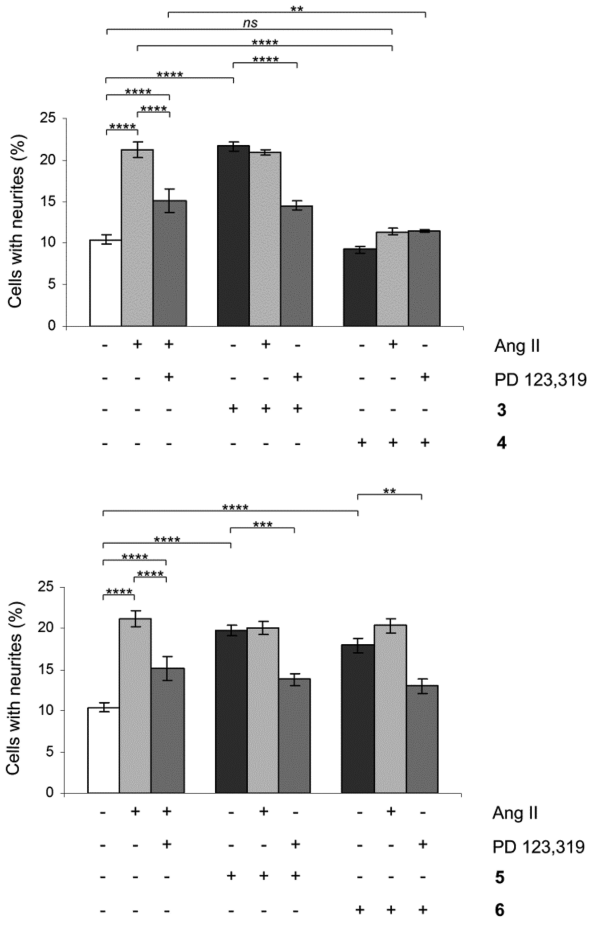

Figure 5. Effect of compounds **3**–**6** on neurite outgrowth in NG108-15 cells. The cells were plated at a cell density of 3.6×10^4 cells/Petri dish (35 mm) and were cultured for 3 days in the absence or presence of 100 nM Ang II, 10 nM **3**, 10 nM **4**, 10 nM **5**, or 10 nM **6** alone or in combination with 10 μM PD 123,319 or 100 nM Ang II. 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 expressed as the percentage of the total number in the micrographs (at least 400 cells according to the experiment). The results are significant according to two-way ANOVA: ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$; *ns* = not significant.

The functionalities are summarized in Table 1 and the neurite outgrowth results are shown in Figure 5.

The biphenyl analogues of **1** and **2**, compounds **3** and **4**, respectively, exhibited a similar biological response both in affinity to and functional activity at the AT_2 receptor, indicating that the biphenyl scaffold is a bioisoster to the thienylphenyl scaffold in this series of compounds. It is worth noting that the antagonist **4** exhibited a better inhibition of Ang II-induced neurite outgrowth compared to PD 123,319, even thought the concentration of **4** was a 1000-fold lower compared to PD 123,319 (10 nM vs 10 μM), as also seen with **2**.[15,16] Ang II stimulation after pretreatment with **4** resulted in neurite outgrowth no longer significantly different compared to untreated cells, while Ang II stimulation after pretreatment with PD 123,319 showed a neurite outgrowth significantly (****, $p < 0.0001$) higher compared to untreated cells. This indicates that compound **4** is a more efficient AT_2 receptor antagonist than PD 123,319 in the cell assay.

Compound **3** that has been previously synthesized and assessed as an AT_2 receptor ligand[5] acts as an agonist (Table 1 and Figure 5). When the substitution pattern for the isobutyl chain of **3** was changed to give **5**, the affinity dropped approximately 10 times, but the agonistic property was retained (Table 1 and Figure 5). When the same structural modification was performed with **4** to deliver compound **6** the affinity was slightly increased, but more interestingly, the antagonistic property of **4** was interconverted to agonism (Table 1 and Figure 5). Thus, independent of the methylene imidazole position, both an agonist and an antagonist could be obtained by moving the isobutyl side chain. This suggests that the structural feature responsible for functionality is the spatial relationship between the imidazole ring and the isobutyl side chain.

When looking at the peptide-activated GPCRs there seems to be a trend that minor structural alterations in small nonpeptidic ligands can interconvert the functional activity, cf. **7** and **8**.[17] Small changes in the effector domain of peptides can frequently also have large impact on functionality, as exemplified by [Ile^8]AngII, which acts as an antagonist at the AT_1 receptor (or partial agonist), while the native [Phe^8]AngII acts as an agonist.[28] Likely it is the same effects on the equilibrium between the active and inactive receptor conformation that we see from these small nonpeptidic ligands.

The presented investigation highlights the importance of the relative position of the methylene imidazole and the isobutyl substituent for functional activity in this class of small molecule AT_2 receptor ligands. Thus, regardless of the position of the methylene imidazole group that previously was proposed to serve as an important determinant for agonism, both agonists and antagonists can be obtained by a short distance migration of the isobutyl group attached to the biphenyl scaffold. This suggests that the structural feature responsible for functionality is the spatial relationship between the imidazole ring and the isobutyl side chain. The data gained from molecular modeling provides a good foundation in the future design of selective AT_2 receptor agonists and antagonists.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedure and analytical data of the synthesized compounds, biological evaluation methods, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

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Notes

The authors declare no competing financial interest.

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