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Original article

The discovery of new potent non-peptide Angiotensin II AT1 receptor blockers: A concise synthesis, molecular docking studies and biological evaluation of *N*-substituted 5-butylimidazole derivatives☆

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ABSTRACT

A convenient and facile synthesis, *in silico* docking studies and *in vitro* biological evaluation of *N*-substituted 5-butylimidazole derivatives as potent Angiotensin II (ANG II) receptor type 1 (AT1) blockers (ARBs) has been reported in the current study. Our efforts have been directed towards the development of an efficient synthetic route allowing the facile introduction of substituents on the imidazole ring. In particular, a series of imidazole based compounds bearing the biphenyl moiety at the $N-1$ position, a halogen atom at the *C*-4 and polar substituents such as hydroxymethyl, aldo or carboxy group at the *C*-2 position were designed and synthesized. These compounds were evaluated for binding to human AT1 receptor and for ANG II antagonism *in vitro* on isolated rat uterus. Among them, 5-butyl-1-[[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole-2-carboxylic acid (**30**) exhibited higher binding affinity compared to the other analogues tested ($-\log IC_{50} = 8.46$). The latter analogue was also found to be the most active in the rat uterotonic test ($pA_2 = 7.83$). Importantly, the binding affinity was higher to that of losartan ($-\log IC_{50} = 8.25$) indicating the importance of carboxy group at the *C*-2 position. Experimental findings are in good agreement with docking studies, which were undertaken in order to investigate ligand/AT1 receptor interactions.

1. Introduction

Hypertension is the leading risk factor for human morbidity and mortality. High blood pressure is associated with cardiovascular disease (CV) and organ damages. However, despite the large number of antihypertensive agents, only a few patients achieve to control their blood pressure. This difficulty is due to the complex pathogenesis of hypertension and related CV [1]. Renin–angiotensin system (RAS) is among the many factors playing role in the pathogenesis of hypertension and CV. The RAS is a hormonal cascade which creates angiotensin peptides and is the main regulator of blood pressure and fluid and electrolyte balance [2–4]. Inhibition of RAS has been established as an effective approach for the treatment of several disorders, including hypertension and congestive heart failure, as well as for the pathogenesis of diabetes and kidney disease

Abbreviations: B(O*i*-Pr)$_3$, triisopropyl borate; *n*-BuLi, *n*-butyllithium; *t*-BuOK, potassium *tert*-butoxide; Et_3N, triethylamine; LTMP, lithium 2,2,6,6-tetramethylpiperidide; $NaBH_4$, sodium borohydride; NOE, Nuclear Overhauser Effect; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium; Et_3SiH, triethylsilane; TBAF·$3H_2O$, tetrabutylammonium fluoride trihydrate; TMP, 2,2,6,6-tetramethylpiperidine; $TMSN_3$, trimethylsilyl azide; Tol, toluene.

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[5]. Research efforts over the last decades have focused on the development of highly selective angiotensin (ANG II) AT1 receptor blockers which provided a more specific blockade of the RAS with virtually no agonist effect and better safety compared to ACE inhibitors [6,7]. Selective blockade of the AT1 receptor may prevent all of the known pathologic effects of ANG II associated with its stimulation, while allowing the positive effects induced by the AT2 receptor.

The discovery of potent and orally active ARBs such as losartan [8,9] and eprosartan [10] has encouraged the development of a large number of similar compounds. Among them, candesartan [11], valsartan [12], irbesartan [13], telmisartan [14], tasosartan [15], olmesartan [16] have been launched and were established as ARBs. In 2011, the U.S. Food and Drug Administration (FDA) approved azilsartan [17,18], which is a newer-generation ARB for the treatment of high blood pressure in adults. Treatment with an ARB has been demonstrated to reduce CV events and heart failure progression as well as to improve renal disease and prevent diabetes and this constitutes the importance of their development [19,20]. The majority of selective ARBs has resulted from the modification or replacement of several pharmacophore groups of losartan. The available data from literature and extensive structure–activity relationship (SAR) studies have shown that the important structural features for potential antihypertensive activity are: (i) a biphenyl moiety at the *N*-1 position of a heterocyclic ring 11; (ii) an acidic functionality such as a tetrazole group or an acidic isostere at the ortho position of the biphenyl group [21–23]; (iii) a short lipophilic alkyl chain substituted on the heterocyclic ring for efficient binding to the receptor [11,24–26]. The DuPont group recommended a lipohilic and electron-withdrawing group such as a halogen atom, CF_3, ethyl or pentafluoroethyl substituents at the *C*-4 of the imidazole ring and a small sized group such as CH_2OH or CO_2H capable of forming a hydrogen bond at *C*-5 [9,16].

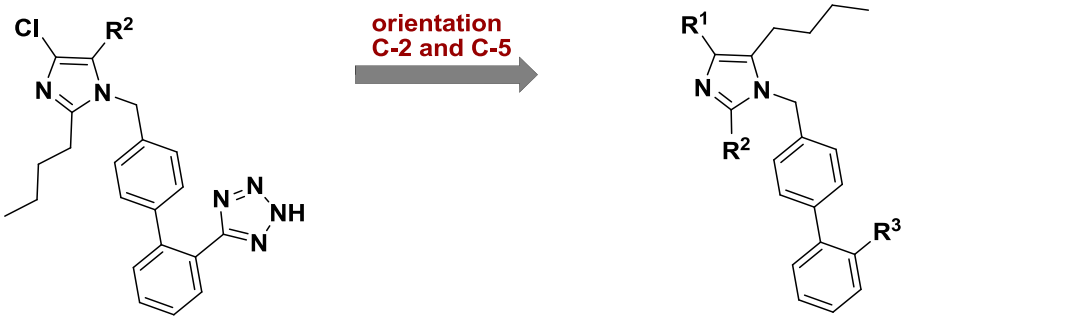
Our work in recent years focused on the SAR studies of ANG II, sarilesin, sarmesin and other synthetic linear and cyclic peptides with antagonistic activity [27–31]. The above data and the continuous demand for antihypertensive agents prompted us to search for new and potent ARBs. Thus, we recently synthesized 1,5-disubstituted imidazole ANG II derivatives found to possess antagonistic properties [32–36]. The design and synthesis of 1,5-disubstituted imidazole ANG II antagonists has demonstrated that reorientation of the substitution pattern at the *C*-2 and *C*-5 positions of the imidazole ring of losartan led to the potent antagonist V8 [35]. In extending these studies, we have designed and performed docking calculations in order to synthesize a variety of synthetic analogues. Indeed, we have developed a new synthetic strategy for compound **21** and we have synthesized a series of structurally related compounds **22**–**24** and **33**, which differ in the orientation of the C-2 and C-5 substituents compared to losartan [26,35,37] (Fig. 1). These compounds bear the butyl chain at the *C*-5 position, the biphenylmethyl group ortho substituted either with tetrazole or its bioisostere carboxy group at the *N*-1 and a halogen atom at the *C*-4 position since the chloro substituent in DuPont series interacts with a lipophilic pocket of the receptor, augmenting affinity [9]. On the other hand, it is well known that losartan is converted by enzymatic oxidation to the active metabolite EXP 3174 [38,39] via the aldehyde EXP 3179 and the former exhibits a 10- to 40-fold higher potency compared to losartan [40,41]. Taking these findings into account, the oxidized forms of **21** were synthesized leading to the aldehyde **29** and the carboxylic acid **30** (Fig. 1).

Our synthetic approach included efficient and regioselective reactions in high yield, allowing the facile introduction of the substituents on the imidazole nucleus. The synthesized analogues were tested for their ANG II-antagonistic activity on rat uterus (pA_2) and their AT1 receptor affinity (IC_{50}) using binding assays.

2. Results and discussion

2.1. Chemistry

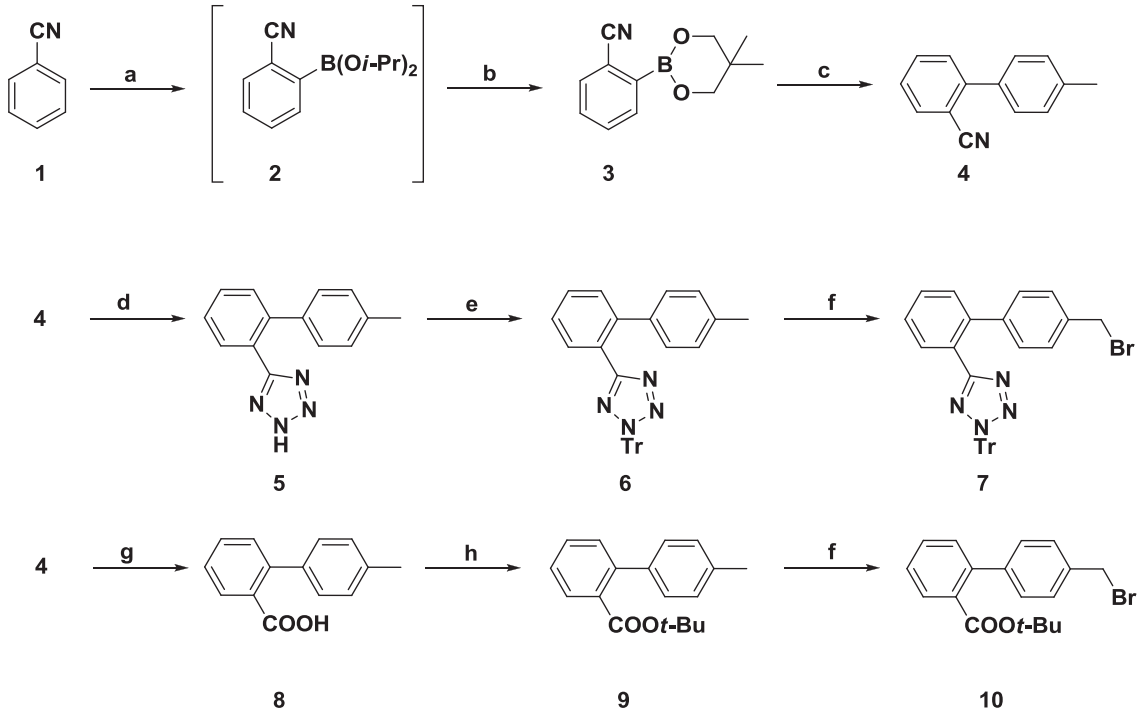
Herein, we describe a concise synthesis of *N*-substituted 5-butylimidazole derivatives (**21**–**24**, **29**, **30** and **33**, Fig. 1). Specifically, the described synthetic method included efficient reactions in high yield, rendering it a general approach for the regioselective substitution on imidazole nucleus in a sequential manner. Furthermore, for the synthesis of 5-(4′-methyl-2-biphenyl)-2*H*-tetrazole (**5**), a three-step method (Scheme 1) was employed in order to minimize the use of expensive reagents and hazardous intermediates via a rapid and mild process [9,42,43]. The intermediates (**4**, **7** and **10**) that were used to introduce the biphenylmethyl



Losartan: R^2=CH_2OH
EXP 3179: R^2=CHO
EXP 3174: R^2=COOH

21: R^1=H, R^2=CH_2OH, R^3=CN_4H
22: R^1=Cl, R^2=CH_2OH, R^3=CN_4H
23: R^1=Br, R^2=CH_2OH, R^3=CN_4H
24: R^1=I, R^2=CH_2OH, R^3=CN_4H
29: R^1=H, R^2=CHO, R^3=CN_4H
30: R^1=H, R^2=COOH, R^3=CN_4H
33: R^1=H, R^2=CH_2OH, R^3=COOH

Fig. 1. Structures of the synthesized compounds compared to losartan and its metabolites EXP 3179 and EXP 3174.



Scheme 1. Synthesis of the alkylating agents **7** and **10**[a]. [a]Reagents and conditions: (a) *n*-BuLi (1.6 M in hexanes), TMP, $B(Oi\text{-}Pr)_3$, THF, −78 °C to rt, 5 h; (b) 2,2-dimethyl-1,3-propandiol, Tol, rt, overnight; (c) 4-bromotoluene, $Pd(PPh_3)_4$, Tol, EtOH, K_2CO_3, 100 °C, 4 h; (d) $TBAF{\cdot}3H_2O$, $TMSN_3$, 120 °C, 36 h; (e) TrCl, Et_3N, CH_2Cl_2, rt, 1 h; (f) NBS, dibenzoylperoxide, CCl_4, reflux, 8 h; (g) 4.5 N HCl, reflux, 12 h; (h) i. oxalyl chloride, CH_2Cl_2, 0 °C to rt, 3 h, ii. *t*-BuOK, THF, 10 °C to rt, 1 h.

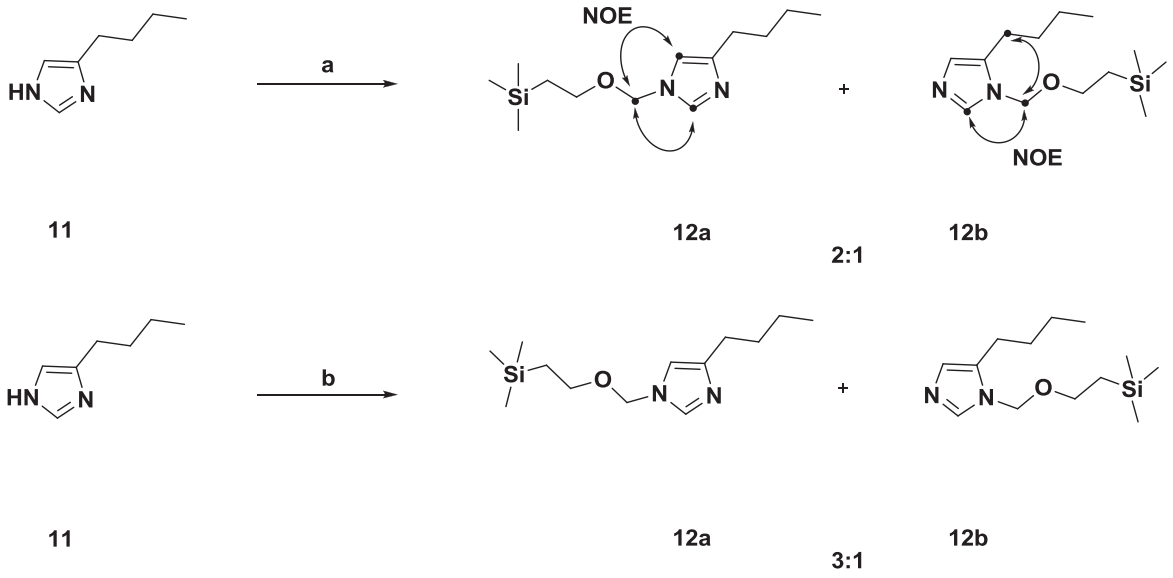
moiety to the imidazole ring were obtained according to reported methods [42–45] as outlined in Scheme 1. The biphenyl nitrile **4** was prepared in two steps from the commercially available ortho-benzonitrile **1** [9,42]. Thus, the synthesis included the conversion of **1** to the intermediate **2** by *in situ* trapping of unstable lithio intermediate, using lithium 2,2,6,6-tetramethylpiperidide (LTMP) as a base in combination with triisopropylborate ($B(Oi\text{-}Pr)_3$) in THF at −78 °C. Subsequent treatment with 2,2-dimethyl-1,3-propanediol afforded the stable arylboronic ester **3** [42]. These mild conditions led to the corresponding air and chromatography stable ortho substituted arylboronic ester **3** in good yield (58%) with excellent purity after recrystallization. The latter was readily converted to the nitrile **4** via Suzuki cross-coupling reaction [42,46,47] using bromotoluene under catalysis with $Pd(PPh_3)_4$ and K_2CO_3 as a base at 100 °C for 4 h in 70% yield. The tetrazole derivative **5** was obtained by [3 + 2] cycloaddition reaction using trimethylsilylazide ($TMSN_3$) as azide source in the presence of $TBAF{\cdot}3H_2O$ under solventless conditions [43] at 120 °C for 36 h in 77% yield.

Protection of the tetrazole ring with the trityl group (Tr) resulted in the corresponding derivative **6**, followed by benzylic bromination to provide **7** as the sole product in good combined yield (2 steps, 64%). On the other hand, acid hydrolysis of the nitrile **4** led to the formation of the carboxylic acid **8** in high yield. Protection of the carboxy group with the *tert*-butyl group was accomplished according to an established procedure [9] using oxalyl chloride and *t*-BuOK, resulting in the ester **9**. Similarly, benzylic bromination of **9** afforded the corresponding derivative **10**.

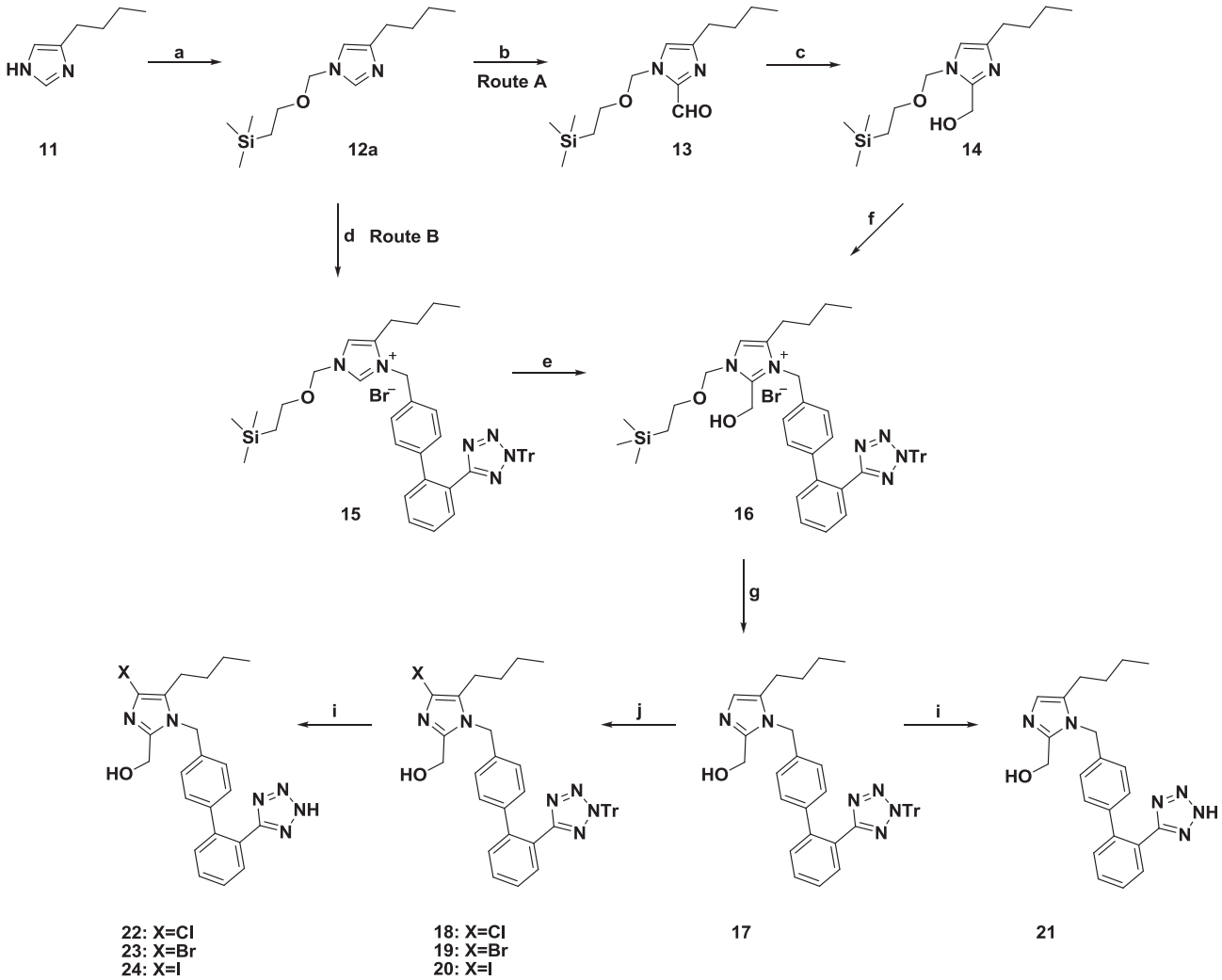
The 2-(trimethylsilyl)ethoxymethyl (SEM) group constitutes a widely used heterocyclic NH protecting group particularly in indoles, pyrroles [48] and imidazoles [49] but is also an excellent directing group for lithiaton at the alpha position [50]. In our case, we have sought an appropriate protecting group at the *N*-1 for ortho directed lithiation at the *C*-2 position with subsequent electrophilic quenching, in combination with regioselective alkylation at the *N*-3 of the 4(5)-butylimidazole (**11**). Therefore, a series of protecting groups was used including the trityl group, the benzyl group, the *N*,*N*-dimethylsulfamoyl group, and the SEM group which proved an excellent choice.

Most protection conditions have employed NaH in DMF to deprotonate acidic NHs, followed by addition of SEM-Cl [51]. Using these standard conditions (SEM-Cl/NaH/DMF) with the imidazole derivative **11**, we found that both regioisomers **12a** and **12b** were formed in high combined yield (84%, Scheme 2). These isomers were separated, characterized by ^1H NMR, ^{13}C NMR and their regiochemistry was unequivocally assigned by 1D NOE experiment. In this case, substitution at the *N*-1 position was favored with a **12a**/**12b** ratio of 2:1 (as indicated by HPLC and ^1H NMR). Therefore, we selected conditions that might improve the regioselectivity in favor of the isomer **12a**. Thus, dicyclohexylmethylamine ($Cyhex_2NMe$) was used as a hindered non-deprotonating base [52] and a substantial improvement of regioselectivity was achieved with a **12a**/**12b** ratio of 3:1 (as indicated by HPLC).

Having the 1-SEM-4-butylimidazole (**12a**) in hand, two synthetic routes were investigated for the synthesis of the key intermediate **16** (Scheme 3). In the first approach (route A), lithiation at the *C*-2 of the imidazole ring **12a** by exposure to *n*-BuLi at −78 °C in anhydrous THF and subsequent quenching with anhydrous DMF from −78 °C to rt for 16 h, afforded the aldehyde **13** in good yield (65%). The extent of lithiation was monitored by quenching a reaction sample with D_2O, resulting in the absence of the H-2 signal at 8.82 ppm, thus proved to be quantitatively by ^1H NMR. Furthermore, the proton aldehyde appeared as a singlet at 9.77 ppm, while the carbon signal appeared at 183.47 ppm. Reduction of **13** in the presence of $NaBH_4$ in MeOH afforded the alcohol **14** (92%). The ^1H NMR spectrum of **14** showed a singlet peak at 4.69 ppm, which is assigned to the hydroxymethyl protons in addition to the absence of the aldehyde proton. Alkylation of **14** at the *N*-3 of the imidazole ring was carried out selectively upon addition of the alkylating agent **7** in MeCN under reflux for 12 h to provide the intermediate salt **16** in poor yield (20%).



Scheme 2. Synthesis and determination of regioisomers **12a** and **12b** by 1D NOE[a]. [a]Reagents and conditions: (a) NaH (powdered, 95%), SEM-Cl, DMF, 0 °C to rt, 2 h; (b) $Cyhex_2NMe$, SEM-Cl, THF, rt, 2 h.



Scheme 3. Synthesis of the target compounds **21**–**24**[a], [a]Reagents and conditions: (a) SEM-Cl, NaH, DMF, 0 °C to rt, 2 h or $Cyhex_2NMe$, SEM-Cl, THF, rt, 2 h; (b) *n*-BuLi (1.6 M in hexanes), THF, DMF, −78 °C to rt, 18 h; (c) $NaBH_4$, MeOH, 0 °C to rt, 2 h (d) **7**, MeCN, reflux, 3 h (e) 37% formalin, diisopropylethylamine, DMF, 85 °C, 1 h (f) **7**, MeCN, 70 °C, 12 h; (g) TBAF (1.0 M in THF), reflux, 8 h; (h) H_2, Pd/C, MeOH, 24 h; (i) 20% TFA/CH_2Cl_2, Et_3SiH, rt, 1 h; (j) NXS (X = Cl, Br, I), DMF.

In order to improve the yield for the formation of **16**, we adopted a second approach (route B), which initially included alkylation of **12a**. Similarly, the reaction was performed in the presence of the alkylating reagent **7** in MeCN under reflux for 3 h, resulting in **15** in 78% yield with high purity after recrystallization. At this point, we were ready to perform the introduction of the hydroxymethyl group at the *C*-2 of the imidazole ring of the alkylated compound **15**. According to our strategy, the hydroxymethylation was promptly carried out in a sealed tube by treatment with diisopropylethylamine and 37% formalin in DMF at 85 °C for 1 h. This reaction proceeded quantitatively as indicated by HPLC, without the need for purification of the hydroxymethylated product **16**. The ^{1}H NMR spectrum of **16** showed the presence of a singlet peak at 4.70 ppm due to the hydroxymethyl protons, while its carbon signal appeared at 48.92 ppm.

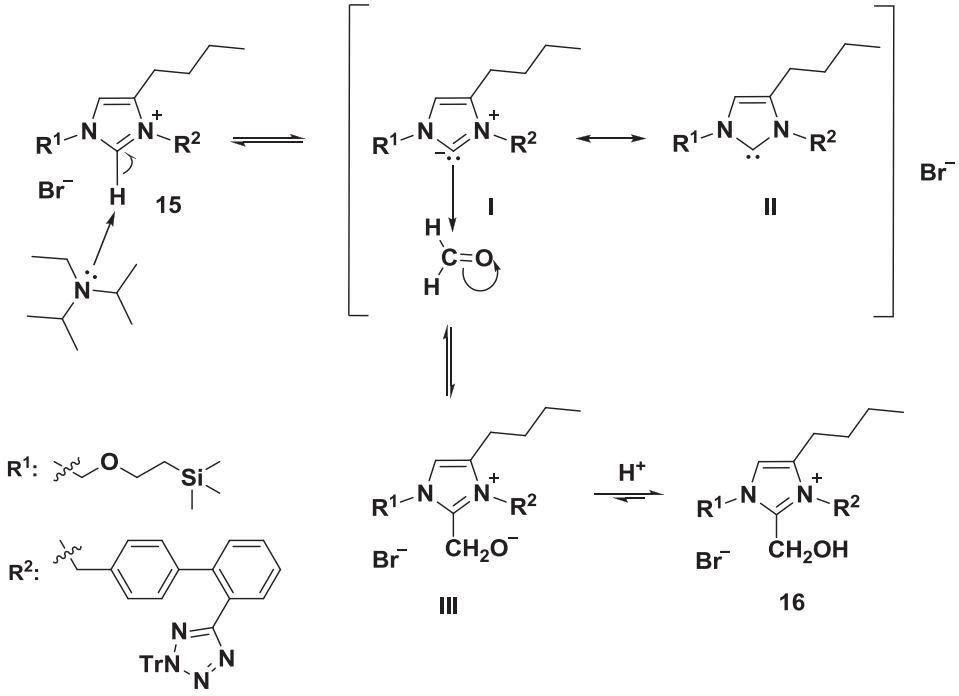
A plausible mechanism for this hydroxymethylation involves the deprotonation by diisopropylethylamine of the more acidic proton at the *C*-2 of the imidazolium salt **15** onto a ylide **I**. The latter species, to which a carbene form **II** is an important resonance contributor, undergoes nucleophilic addition to formaldehyde (Scheme 4) affording the hydroxymethylated compound **16**.

The SEM group of the intermediate salt **16** was then cleaved by treatment with 1 M TBAF in THF under reflux resulting in the alcohol **17**. Halogenation of **17** at the *C*-4 position of the imidazole ring with the appropriate *N*-halosuccinimide (NXS, X = Cl, Br, I) in DMF afforded the corresponding halogenated analogues **18**–**20**. The tetrazole group of **17**–**20** was promptly deprotected upon addition of 20% trifluoroacetic acid (TFA) in CH_2Cl_2 in the presence of Et_3SiH as scavenger to obtain the final compounds **21**–**24** in 79–85% yield. The ^{1}H NMR spectra of the final halogenated analogues **22**–**24** showed the absence of the H-4 signal of the imidazole ring at 7.36 ppm, appearing at **21**. Not surprisingly, the ^{13}C NMR spectra showed an upfield trend in *C*-4 chemical shifts produced by bromine and iodine atoms for **23** and **24**, at 111.45 and 102.43 ppm, respectively, due to the "heavy atom effect", while the *C*-4 in the chloro derivative **22** was deshielded at 124.43 ppm.

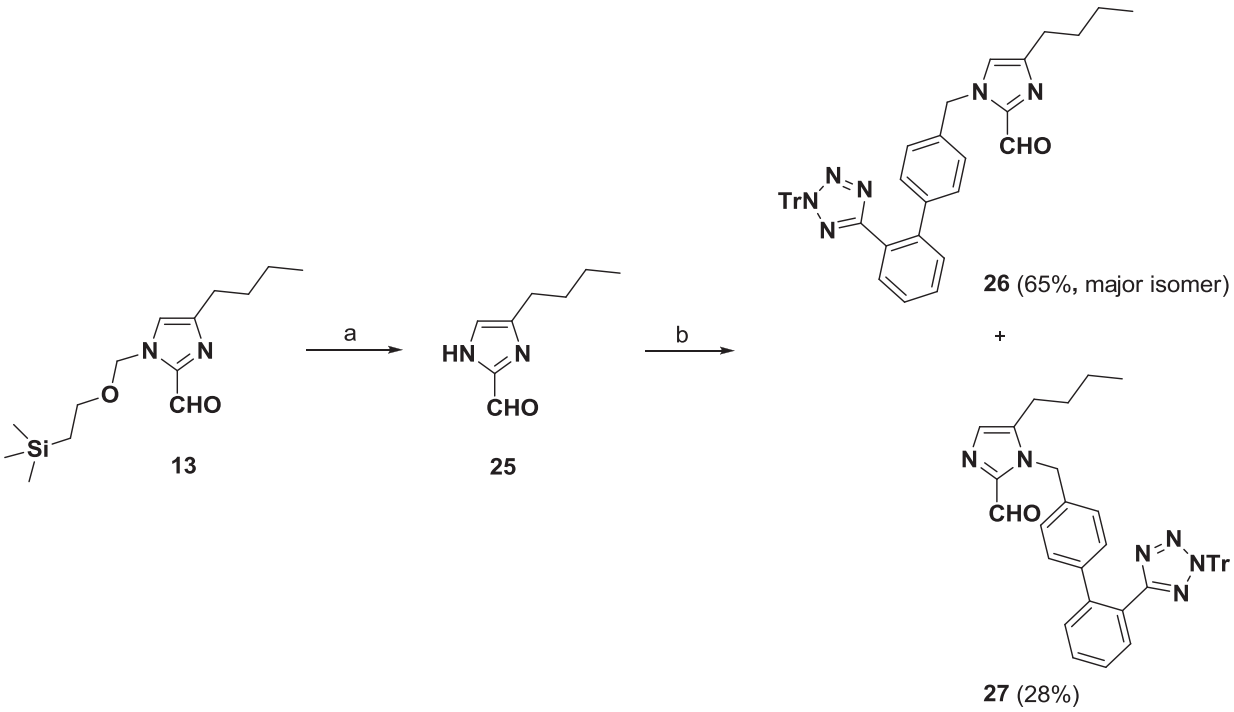
Having proven that losartan is *in vivo* metabolized to the main active metabolite EXP 3174 via the EXP 3179, we then set out to synthesize **29** and **30**, presumably as metabolites of the most potent hydroxymethyl derivative **21**. Scheme 5 depicts the initial attempt towards the synthesis of **27** as a precursor of the final aldehyde **29**. Thus, treatment of **13** with 3 N HCl in MeOH led to the cleavage of the SEM group to afford the intermediate **25**, which was alkylated upon addition of **7** in the presence of K_2CO_3 in DMF. The expected *N*-alkylated regioisomers **26** and **27** were separated by flash column chromatography and their regiochemistry was confirmed by 2D NOESY experiment. This task was proved rather simple, however, the desired aldehyde **27** was obtained as the minor regioisomer (28%) with **26**/**27** ratio of ca. 2.5:1 (as indicated by HPLC).

Therefore, our chosen synthetic sequence for the preparation of the aforementioned **29** and **30** is depicted in Scheme 6 (Method A). Hence, the alcohol **17** was converted to the aldehyde **27** via Swern oxidation [53,54] by treatment with dimethyl sulfoxide (DMSO) and oxalyl chloride in CH_2Cl_2 at −78 °C. Subsequently, addition of Et_3N at −60 °C led to **27** in excellent yield (92%). The proton signal at 9.77 ppm was attributed to the aldehyde in addition to the carbon signal at 181.91 ppm. The latter was subjected to Lindgren oxidation by means of $NaClO_2$ and NaH_2PO_4 in the presence of 2-methyl-2-butene as chlorine scavenger [55,56] to afford the carboxylic acid **28**, quantitatively. Detritylation of the tetrazole group of the oxidized intermediates **27** and **28** was accomplished by treatment with formic acid in Et_2O, resulting in the final compounds **29** and **30**, respectively. Alternatively, the carboxylic acid **30** was also obtained in one step via oxidation of the final alcohol **21** with $KMnO_4$ (Scheme 6, Method B) in the presence of 18-crown-6 in H_2O/acetone in 74% yield. The absence of the aldehyde proton signal, as well as the carbon signal at 172.10 ppm of **30**, unequivocally confirmed the introduction of the carboxy group.

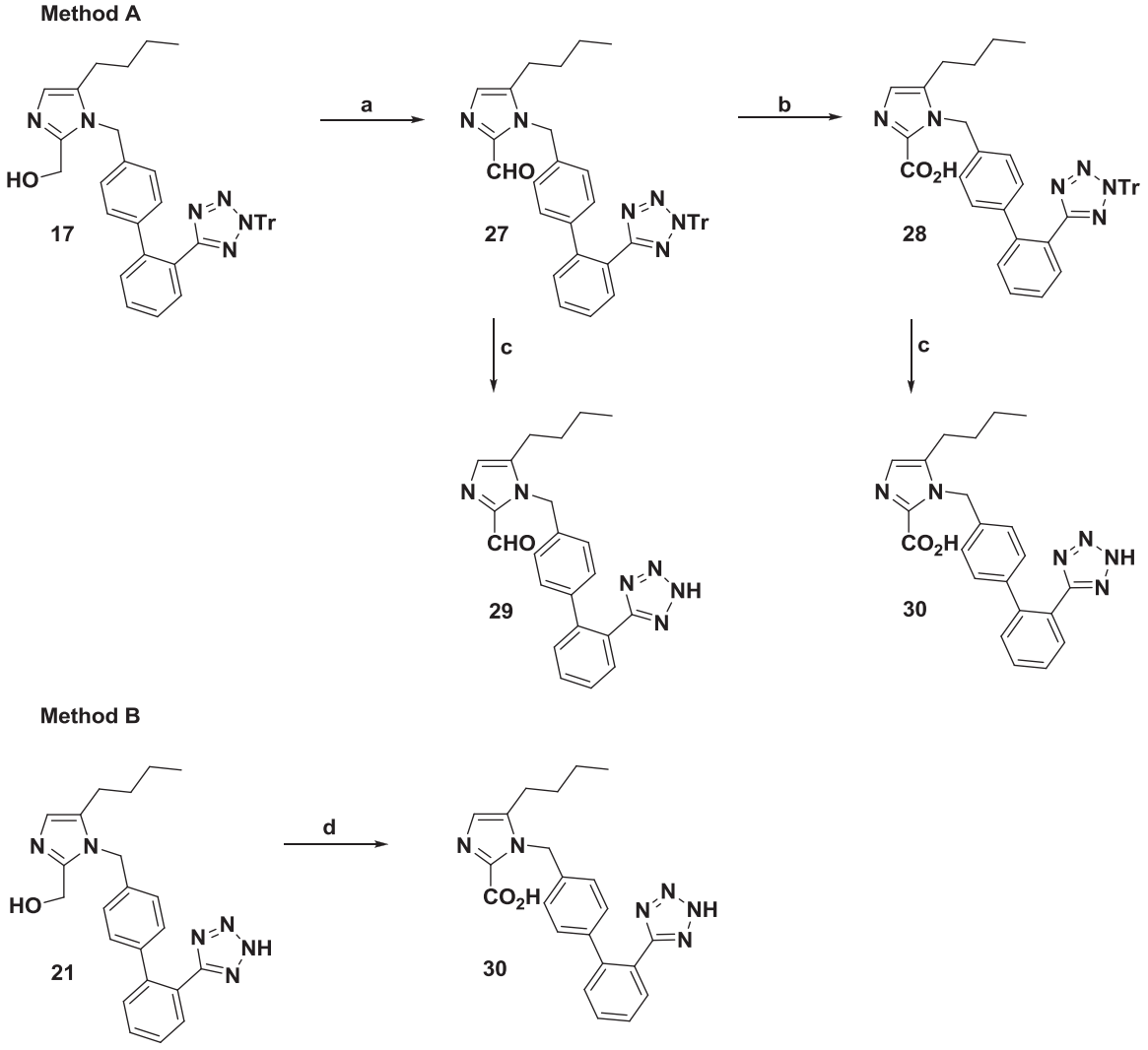
Finally, employing the previously described method, the preparation of the compound **33** where the tetrazole group is replaced



Scheme 4. Proposed mechanism of hydroxymethylation at the *C*-2 position of the imidazolium salt **15**.



Scheme 5. Synthesis of the aldehyde **27**[a]. [a]Reagents and conditions: (a) 3 N HCl, EtOH, 60 °C, 3 h; (b) **7**, K_2CO_3, DMF, rt, 18 h.



Scheme 6. Synthesis of the aldehyde **29** and the carboxylic acid **30**[a]. [a]Reagents and conditions: (a) DMSO, oxalyl chloride, CH_2Cl_2, −78 °C, 1 h then Et_3N, −60 °C to rt, 1 h; (b) NaH_2PO_4, $NaClO_2$, 2-methyl-2-butene, *t*-BuOH/H_2O (1:1), rt, 1 h; (c) formic acid/Et_2O (3:2), rt, 2 h; (d) $KMnO_4$, 18-crown-6, acetone/H_2O (1:1).

by the bioisostere carboxy group commenced with alkylation at the *N*-3 of **12a** by treatment with **10** in MeCN, resulting in the intermediate salt **31** (Scheme 7). The latter was subjected to hydroxymethylation at *C*-2 of the imidazole ring of **31** in the presence of diisopropylethylamine and 37% formalin in DMF at 85 °C for 1 h to afford **32** in 93% yield. Finally, removal of the *tert*-butyl group by means of 20% TFA in CH_2Cl_2 and Et_3SiH led to the carboxy compound **33** in 82% yield.

2.2. Pharmacology

The pharmacological evaluation of the synthesized ARBs was determined by means of their binding affinities for the human AT1 receptor (using losartan as control), as well as their ability to inhibit the contractility effect of ANG II in isolated rat uterus. The binding affinities ($-\log IC_{50}$) were determined from competition experiments performed under equilibrium conditions in membranes from HEK 293 cells stably expressing the AT1 receptor and using as a radioligand the [^{125}I–Sar^1–Ile^8] ANG II. As shown in Figs. 2 and 3, all analogues evaluated herein bound to AT1 receptor in a concentration dose-dependent manner similar to losartan. The binding affinity of the alcohol **21** ($-\log IC_{50} = 7.52$) for the AT1 receptor was only 5.5-fold lower than that of losartan ($-\log IC_{50} = 8.25$, Table 1) as it was also indicated in our previous work [35]. An interesting observation is that the introduction of a halogen atom (Cl, Br, I) at the *C*-4 of the imidazole ring affording **22**–**24**, exhibited a sharp drop of affinity ($-\log IC_{50} = 6.20, 6.93, 6.35$, respectively, Figs. 2 and 3, Table 1). These results indicate that a lipophilic halogen substituent at the *C*-4 position is not beneficial in terms of binding affinity. On the other hand, the conversion of **21** to the aldehyde **29** did not significantly alter the binding affinity ($-\log IC_{50} = 7.62$, Fig. 3, Table 1). Interestingly, further oxidation of the aldo group of

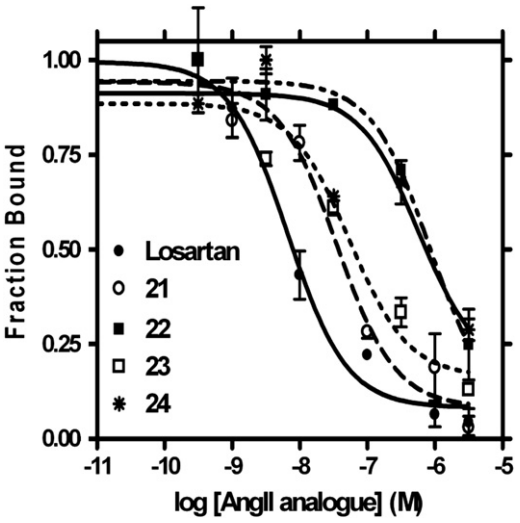
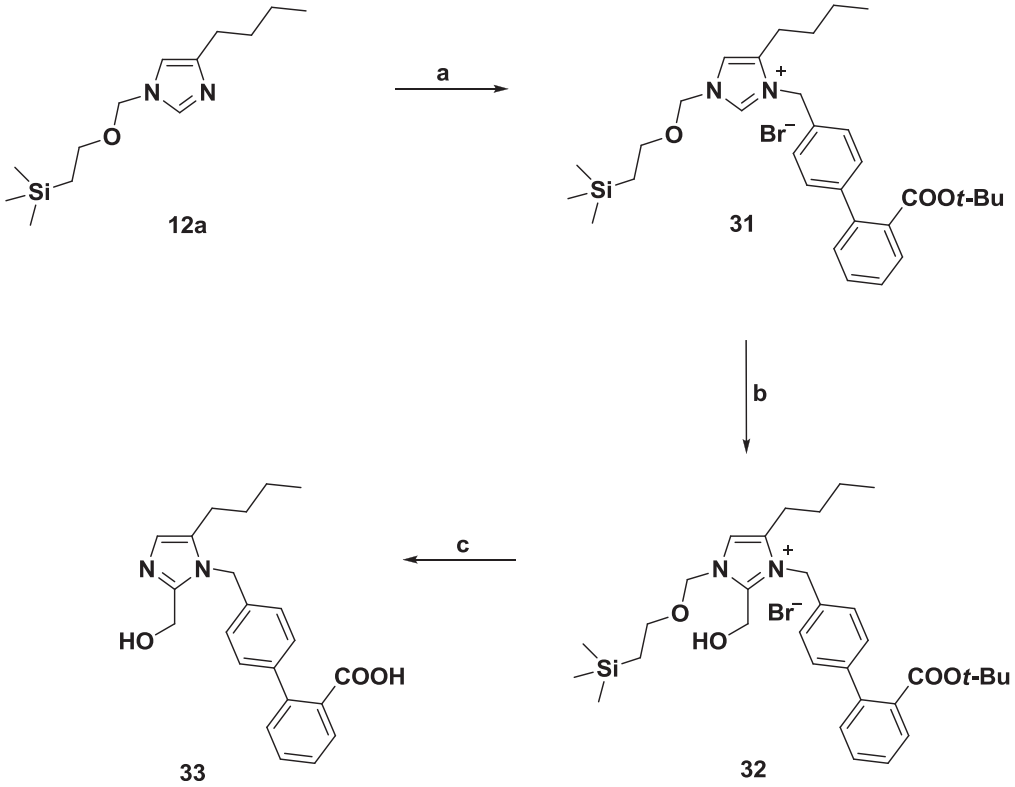


Fig. 2. Competition binding isotherms of ANG II analogues to human AT1 receptor. Competition of [^{125}I–Sar^1–Ile^8] ANG II specific binding by increasing concentrations of ANG II analogues, losartan, and compounds **21**, **22**, **23** and **24** was performed, as described in Experimental section membranes from HEK 293 cells stably expressing the human AT1 receptor. The means and S.E. are shown from 2 to 5 different experiments. The data were fit to a one-site competition model by nonlinear regression and the $-\log IC_{50}$ values were determined as described in Experimental section.

29 resulted in the carboxylic acid **30**, revealing a significant increase in affinity which was higher than that of losartan ($-\log IC_{50} = 8.46$ and 8.25, respectively, Fig. 3, Table 1). In contrast, replacement of the tetrazole group into **21** with bioisostere carboxy group resulted in the analogue **33** having 44-fold lower affinity than losartan ($-\log IC_{50} = 6.61$, Fig. 3, Table 1). As expected, these data illustrate the



Scheme 7. Synthesis of the carboxy compound **33**[a]. [a]Reagents and conditions: (a) **10**, MeCN, reflux, 3 h; (b) 37% formalin, diisopropylethylamine, DMF, 85 °C, 1 h; (c) 20% TFA in CH_2Cl_2, Et_3SiH, rt, 1 h.

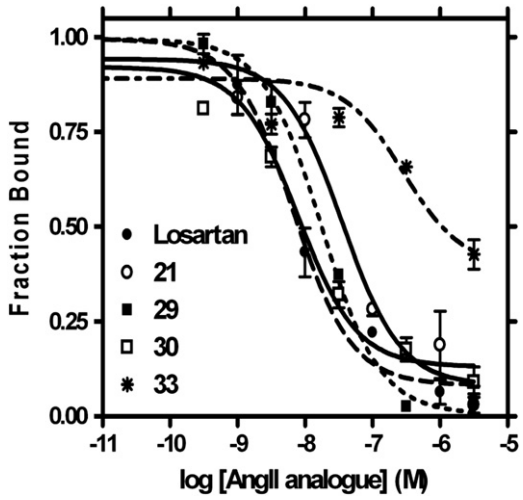


Fig. 3. Competition binding isotherms of ANG II analogues to human AT1 receptor. Competition of [^{125}I–Sar^{1}–Ile^{8}] ANG II specific binding by increasing concentrations of ANG II analogues, losartan, and compounds **21**, **29**, **30** and **33** was performed, as described in Experimental section, on membranes from HEK 293 cells stably expressing the human AT1 receptor. The means and S.E. are shown from 3 to 4 different experiments. The data were fit to a one-site competition model by nonlinear regression and the −log IC_{50} values were determined as described in Experimental section.

importance of tetrazole as pharmacophore group for binding to the AT1 receptor.

Next the synthesized compounds were tested in rat uterotonic *in vitro* test for their ability to inhibit the contractions evoked by ANG II as described in Experimental section. The results obtained are summarized in Table 1. As can be seen, the most active compounds of this series were **29** and **30** (pA_2 = 7.76 and 7.83, respectively). Their activity was only about 2 times lower than that of losartan (pA_2 = 8.25).

In the case of the most active compounds **29** and **30**, their specificity for the AT1 receptor was also tested. Their effect was found specific, as they did not influence the standard cumulative dose response curves of bradykinin and oxytocin using even 1000 times higher concentration than that corresponding to pA_2 which completely diminished the response to very high doses of ANG II.

Conclusively, the pharmacological results of this study suggest that the aldo as well as the carboxy group at the *C*-2 of the imidazole ring was favorable for the interaction with AT1 receptor, with the carboxy compound **30** to show slightly higher affinity. In Fig. 4, SAR data of the synthesized compounds **21**–**24**, **29**, **30** and **33** are summarized.

Table 1
In vitro activities of synthesized compounds (competition binding studies were performed on membrane preparations from HEK 293 cells stably expressing human AT1 receptor. The −log IC_{50} values were obtained by fitting the data from the competition studies to a one-site competition model by nonlinear regression. The mean ± S.E. values are from 2 to 5 independent experiments. The IC_{50} is derived from the mean value of log IC_{50}).

Compound	pA_2	IC_{50} (nM)	−log IC_{50}
Losartan	8.25 ± 0.13	6.0	8.25 ± 0.06
21	7.97 ± 0.07	30.0	7.52 ± 0.16
30	7.83 ± 0.20	3.0	8.46 ± 0.29
29	7.76 ± 0.47	24.0	7.62 ± 0.13
23	7.58 ± 0.15	117.0	6.93 ± 0.16
24	7.35 ± 0.24	446.0	6.35 ± 0.11
22	6.98 ± 0.19	634.0	6.20 ± 0.13
33	6.19 ± 0.26	245.0	6.61 ± 0.15

2.3. Theoretical calculations—molecular docking studies

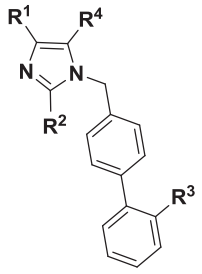
Molecular docking results are tabulated at Table 2 together with experimental activities. Losartan and **30** showed higher (absolute values) Glide XP docking scores in accordance with experimental analyses which showed that these analogues have high binding affinity to the AT1 receptor. Although interaction energy trend for the halogen derivatives are similar with the *in vitro* pIC_{50} values (**23** > **22** > **24**), calculated docking for compound **23** is overestimated. In Fig. 5 atomistic details of the docking complexes from top docking poses was represented for compound **30**. Corresponding amino acid residues are His256, Gln257, Asn200, Tyr113, Trp253, Thr260, Phe182, Lys199, Ile197, Val169, Gly196, Val264 and Phe204 for docking complex of **30** (Fig. 5).

Since compound **23** showed high docking score, this docking complex was also analyzed (Fig. 6). Close contacts are formed by Asn200, Phe261, Gly196, Lys199, Tyr184, Pro192, Val264 and Leu265 for this docking complex. Table 2 also includes Prime energy components and IFD scores, showing the steric and electronic contributions of ligand/target complexes. In Fig. 7 are shown the docking results for **22**–**24**.

3. Conclusions

In this article, a series of *N*-substituted 5-butylimidazole derivatives has been synthesized, via a convenient and facile synthesis and evaluated for their *in vitro* antagonistic activities on ANG II AT1 receptor. In this series, the most potent analogue was found to be **30** bearing the carboxy group at the *C*-2 position. Previous work has shown that compound **21** is slightly less potent than losartan and structurally identical to losartan except that butyl and hydroxymethyl groups are interchanged though is lacking the chlorine atom [35]. Thus, the polar substituent CH_2OH at the *C*-2 is favorable for the antagonistic activity. *In vitro* results showed that generally substitution at the *C*-4 of **21** with halogens resulting in **22**–**24** led to significant decrease of activity. The latter points out that the lipophilic halogen substituents (Cl, Br, I) are unfavorable for the binding affinity for this class of analogues. Molecular docking calculations showed that replacement of a hydrogen atom at the *C*-4 of the imidazole ring with halogens would have not serious detrimental effect except for bromine atom whose its interaction energy is overestimated. To follow a similar strategy with losartan, the compounds **29** and **30** were synthesized as oxidized forms of **21**. Thus, **29** structurally resembles losartan derivative EXP 3179 and **30** its active metabolite EXP3174. In our case, both the oxidized compounds **29** and **30** showed similar antagonistic activity in the uterotonic test as the compound **21** even if the binding affinity of **30** was one order of magnitude higher. This intriguing result showed clearly that the interchange between butyl and oxidized forms of hydroxymethyl group can lead to potent analogues. Interestingly, **30** lacks the chlorine atom but still retains equipotency with losartan. Moreover, replacement of the tetrazole ring by its bioisostere CO_2H resulting in **33**, was detrimental for activity as it was also shown with losartan series.

The Glide XP docking results are in good agreement with experimental findings except for the prediction of interaction energy of brominated analogue **23**. As seen in the Prime energy calculations, **23** has the lowest and overestimated Coulomb energy compared to other analogues. Since Prime Energy mainly determines the IFD score, they cannot be discriminated based on their interaction energy profiles. Nevertheless, considering the limitations of molecular docking algorithms (i.e. simplified approaches of potential solvent effects and entropy), correct estimation of interaction energies for all docked structures except one; encourages us to use molecular docking algorithms in binding energy predictions for this class of molecules.



Compound	R^1	R^2	R^3	R^4	pA_2	$-logIC_{50}$
Losartan	Cl	*n*-butyl	CN_4H	CH_2OH	8.25±0.13	8.25 ± 0.06
21	H	CH_2OH	CN_4H	*n*-butyl	7.97±0.07	7.52 ± 0.16
30	H	COOH	CN_4H	*n*-butyl	7.83±0.20	8.46 ± 0.29
29	H	CHO	CN_4H	*n*-butyl	7.76±0.47	7.62 ± 0.13
23	Br	CH_2OH	CN_4H	*n*-butyl	7.58±0.15	6.93 ± 0.16
24	I	CH_2OH	CN_4H	*n*-butyl	7.35±0.24	6.35 ± 0.11
22	Cl	CH_2OH	CN_4H	*n*-butyl	6.98±0.19	6.20 ± 0.13
33	H	CH_2OH	COOH	*n*-butyl	6.19±0.26	6.61 ± 0.15

Fig. 4. SAR data of the synthesized compounds.

In conclusion, the new series of analogues not only show that like losartan interact with the AT1 receptor in a similar fashion in the AT1 pocket of the active site but also follow the same trend as its analogues. However, the only difference is that these analogues do not demand the presence of lipophilic halogen atom at the *C*-4 position of the imidazole ring. Finally, the data clearly demonstrate the importance of having a carboxylic acid at the *C*-2 as in **30** in order to enhance the binding affinity to the AT1 receptor, thereby contributing to potentiation of activity (Fig. 4). The efficient, short-step, high yield synthesis of 1,5-disubstituted ARBs, the properly effective reorientation of the pharmacophore groups and the introduction of a carboxy group at the *C*-2 position resulting in strong ANG II blockers, is a contribution to the field and may open new avenues in antihypertensive drug therapy.

4. Experimental section

4.1. General

Starting materials were obtained by Aldrich and used as received. All reactions involving air sensitive reagents were performed under argon atmosphere using syringe-septum cap techniques. All reactions were carried out in anhydrous solvents and distilled according to literature. Melting points were determined with a Stuart SMP 10 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX spectrometer at 400.13 MHz and 161.76 MHz, respectively. The 1D NOE spectra were recorded on a Bruker 600 MHz spectrometers. Chemical shifts are reported in units, parts per million (ppm)

Table 2
Docking results of synthesized compounds compared to losartan.

Compound	Glide XP score (kcal/mol)	IFD score (kcal/mol)	Prime Coulomb energy (kcal/mol)	Prime Covalent energy (kcal/mol)	Prime VDW energy (kcal/mol)	Prime solv. SA	Prime solv. GB	Prime energy (kcal/mol)
Losartan	−12.114	−565.712	−9668.993	2510.356	−1547.833	0.398	−2365.894	−11072.0
21	−11.464	−565.848	−9576.544	2474.498	−1526.175	2.807	−2462.276	−11087.7
30	−11.103	−565.583	−9438.741	2489.792	−1519.70	2.169	−2623.110	−11089.6
29	−10.691	−564.090	−9545.308	2479.365	−1544.166	2.241	−2460.108	−11068.0
23	−12.894	−567.479	−9706.724	2501.439	−1523.437	−0.732	−2362.244	−11091.7
24	−10.410	−565.233	−9570.051	2472.930	−1542.117	2.755	−2459.244	−11096.5
22	−10.912	−565.325	−9568.922	2479.481	−1552.117	1.527	−2448.225	−11088.3
33	−9.148	−565.085	−9538.172	2481.973	−1527.480	5.142	−2540.213	−11118.8

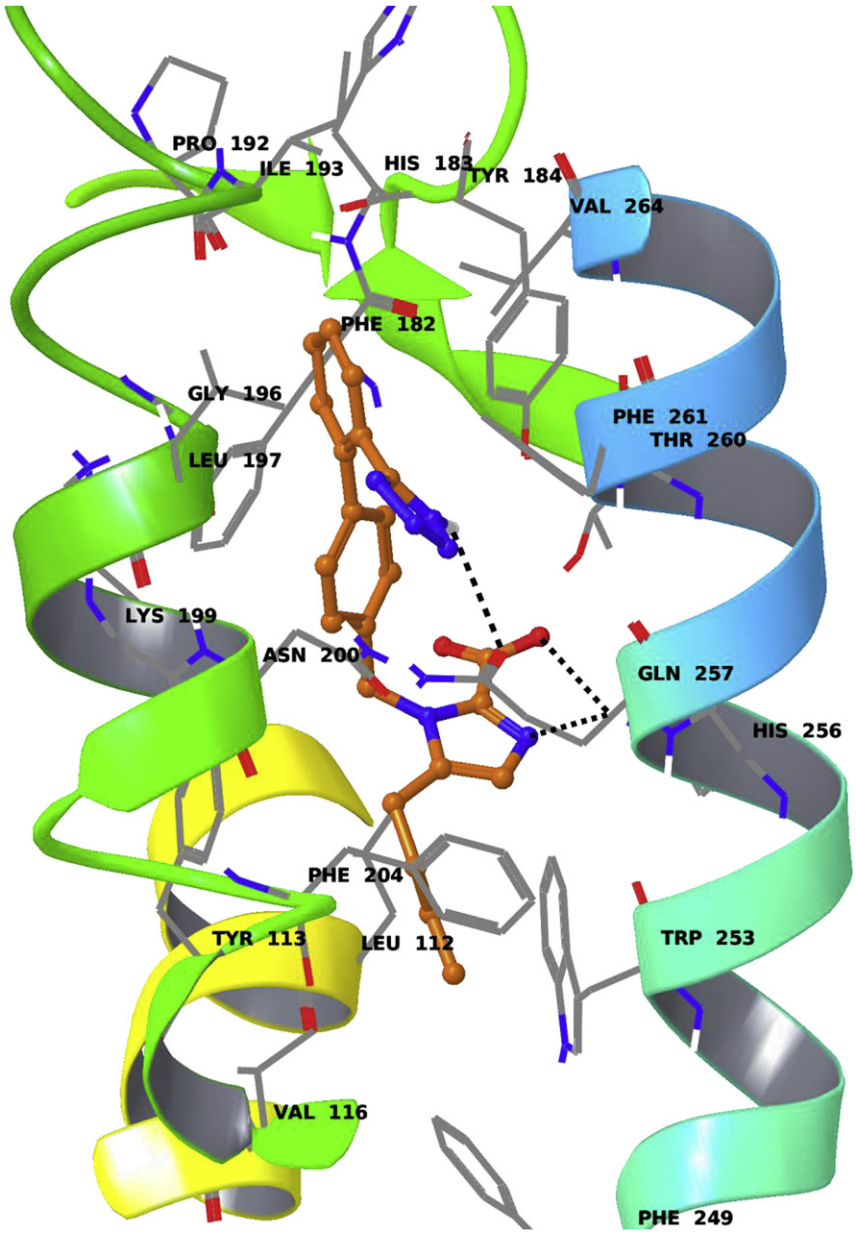


Fig. 5. Top docking pose of **30** at the active site of the receptor.

downfield from tetramethylsilane (TMS) and coupling constants (*J*) are given in Hertz (Hz). HPLC analysis was performed on an Alliance Waters 2695 equipped with a Waters 2996 Photodiode Array Detector UV vis, using the XBridge C18 column (4.6 × 150 mm, 3.5 μm) as stationary phase and a gradient of H_2O/MeCN bothcontaining 0.08% TFA as mobile phase. Electrospray-ionization mass spectra (ESI-MS) were obtained on a UPLC (ultra performance liquid chromatography) equipped with SQ detector Acquity™ by Waters. Analytical TLC was performed on silica gel 60 F_{254} plates (Merck, Germany) and visualized by UV irradiation, iodine and Brady's reagent. Purification of compounds **12a** and **12b** was performed by Waters preparative HPLC equipped with Waters Prep LC Controller and Photodiode Array Detector 2996 using SunFire Prep C18 column (50 × 100 mm) with 5 μm packing material. Separation was achieved using H_2O/MeCN as a mobile phase with a stepped linear gradient from 40% MeCN to 100% MeCN in 50 min with a flow rate of 9 mL/min.

4.2. Chemistry

4.2.1. 2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-benzonitrile (**3**) [42]

To a solution of TMP (3.28 mL, 19.4 mmol) in anhydrous THF (20 mL), *n*-BuLi (1.6 M in hexanes, 11.7 mL, 18.8 mmol) was added at −10 °C and the resulting mixture was stirred for 10 min. Then, $B(Oi\text{-}Pr)_3$ (5.50 mL, 25 mmol) was added dropwise at −78 °C and stirred for additional 5 min before benzonitrile **1** (1.28 mL, 12.5 mmol) was added via a syringe in a single portion and the reaction mixture was stirred at −78 °C for 2 h. The solution was left to warm to rt while being stirred for 3 h and then quenched with saturated aqueous NH_4Cl (60 mL). The resulting mixture was extracted with EtOAc (3 × 70 mL), the combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The intermediate product **2** was dissolved in anhydrous Tol (50 mL) and 2,2-dimethyl-1,3-propandiol (1.57 g, 15 mmol) was added and then stirred overnight at rt. The organic phase was washed with H_2O

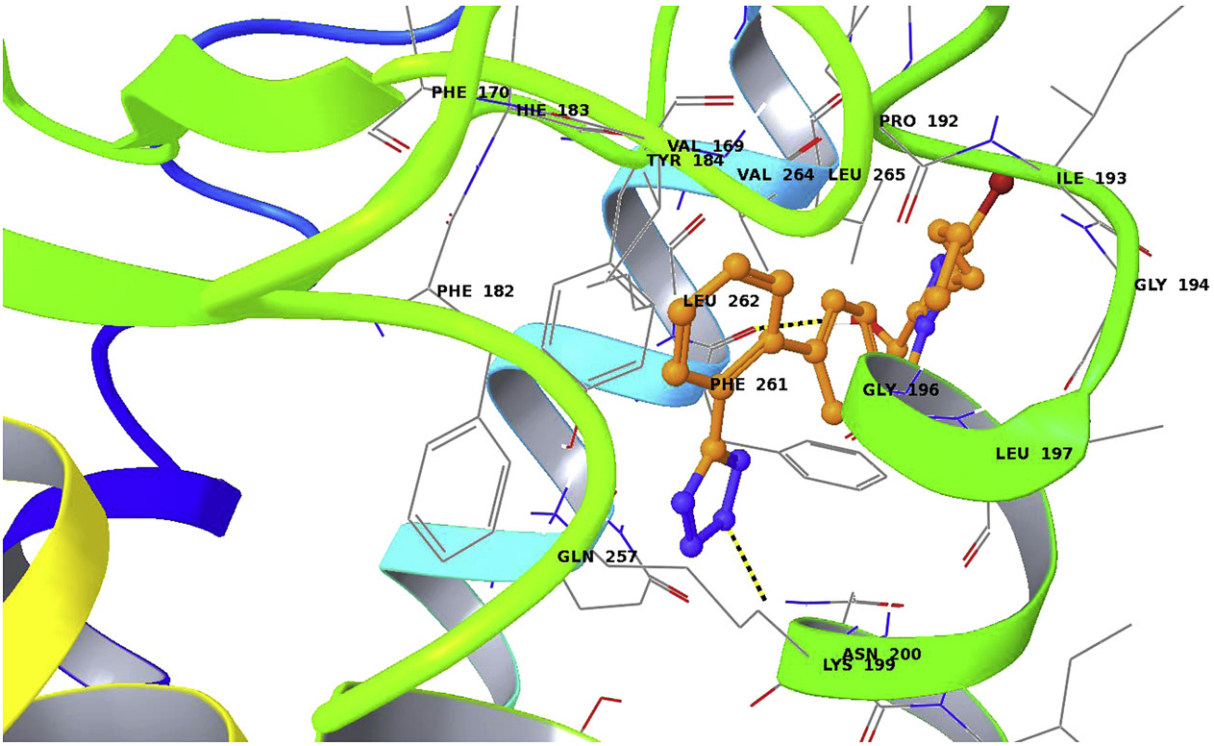


Fig. 6. Top docking pose of **23** at the active site of the receptor.

(3 × 30 mL) and the aqueous extracts were washed with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2-phase was washed with H_2O (1 × 30 mL), combined with Tol extract, dried (Na_2SO_4) and concentrated in vacuo. Recrystallization of the crude product from heptane afforded pure **3** (58%, 2 steps) as a white crystalline solid: M.p. 109–111 °C; ESI-MS (*m*/*z*): 216.64 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, 1H, J = 7.5 Hz), 7.68 (d, 1H, J = 7.5 Hz), 7.54 (td, 1H, J = 7.5 Hz, 1.0 Hz), 7.48 (td, 1H, J = 7.5 Hz, 1.0 Hz), 3.83 (s, 4H), 1.05 (s, 6H) ppm; ^{13}C

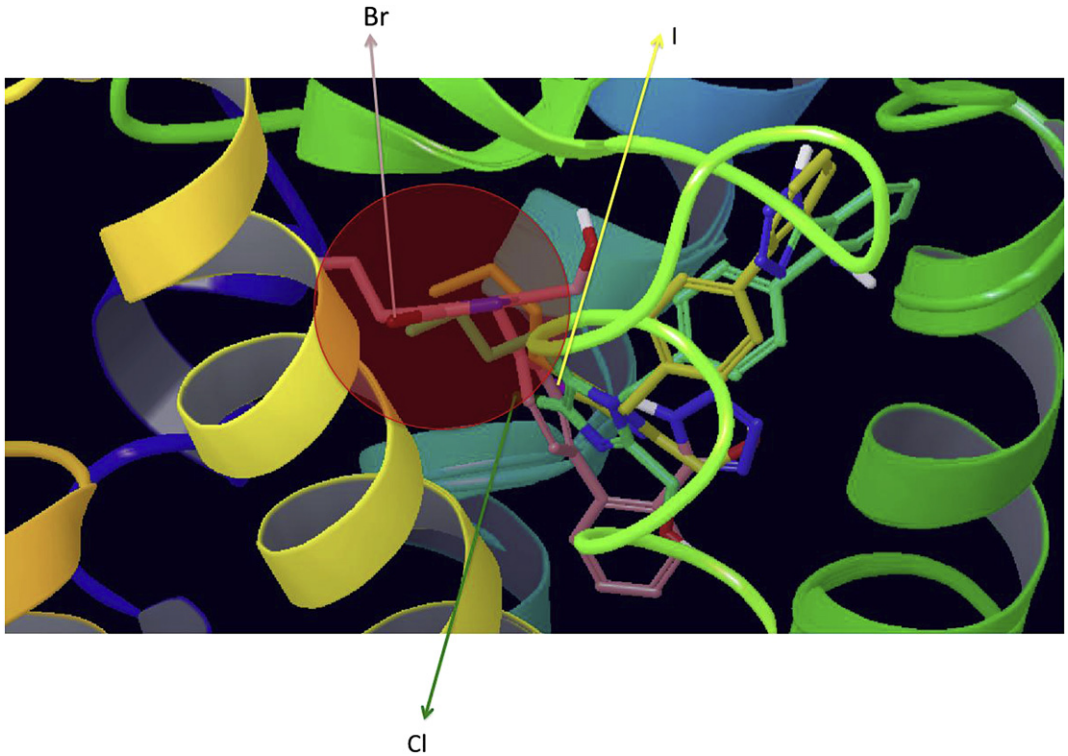


Fig. 7. Superimposition of top docking poses of **22**, **23** and **24** at the active site of the receptor. The arrows show the positions of the halogens (highlighted region). Clearly, chlorine and iodine are positioned in a spatial vicinity while bromine is localized in a different position.

NMR (160 MHz, $CDCl_3$): δ 135.07, 133.64, 131.44, 130.47, 119.63, 116.56, 72.49, 31.84, 21.83 ppm.

4.2.2. 4′-methyl-2-biphenylcarbonitrile (**4**) [9,42]

To a solution of the boronic ester **3** (0.80 g, 3.72 mmol) in Tol (30 mL) and EtOH (3 mL) under argon atmosphere, 4-bromotoluene (0.50 mL, 4.10 mmol), 2 M K_2CO_3 (3 mL) and $Pd(PPh_3)_4$ (3 mol%, 0.19 g) were successively added and the resulting reaction mixture was stirred at 100 °C for 4 h. After cooling the mixture to rt, saturated aqueous NH_4Cl (70 mL) was added, followed by extraction with EtOAc (3 × 70 mL), drying (Na_2SO_4) and evaporation in vacuo. Flash column chromatography (hexanes:EtOAc, 3:1) afforded nitrile **4** (70%) as a white solid: M.p. 50–52 °C; R_f 0.52 (hexanes:EtOAc, 1:4); t_R 15.15 min (30% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 194.06 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.51 (m, 1H), 7.50–7.48 (m, 1H), 7.42–7.34 (m, 4H), 7.22 (d, 2H, *J* = 7.6 Hz), 2.37 (s, 3H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 145.53, 138.69, 135.27, 133.71, 132.76, 129.97, 129.45, 128.61, 127.27, 118.88, 111.18, 21.25 ppm.

4.2.3. 5-(4′-methyl-2-biphenyl)2H-tetrazole (**5**) [9,42,43]

To a sealed tube were successively added TBAF $3H_2O$ (0.35 g, 1.10 mmol), **4** (0.42 g, 2.20 mmol) and $TMSN_3$ (0.38 g, 3.30 mmol) and the resulting mixture was heated at 120 °C for 36 h. The resulting reaction mixture was extracted with EtOAc (30 mL) and TBAF was removed by washing the organic phase with 1 M HCl aqueous solution (3 × 10 mL). The organic extract was dried (Na_2SO_4), filtered and concentrated in vacuo. Recrystallization from diisopropyl ether furnished pure **5** (77%) as a white solid: M.p. 145–147 °C; R_f 0.57 ($CHCl_3$:MeOH, 9:1); t_R 11.54 min (30% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 237.0 [MH]; ^{1}H NMR (400 MHz, CD_3OD): δ 7.69–7.64 (m, 2H), 7.56–7.52 (m, 2H), 7.13 (d, 2H, *J* = 7.8 Hz), 7.00 (d, 2H, *J* = 7.8 Hz), 2.33 (s, 3H) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 155.42, 142.17, 137.43, 136.24, 131.05, 130.39, 130.17, 128.76, 127.27, 122.83, 19.72 ppm.

4.2.4. N-(trityl)-5-[4′-(methyl)biphenyl-2-yl]tetrazole (**6**)

Prepared according to reported procedure [9]. M.p. 161–163 °C; R_f 0.48 (hexanes:EtOAc, 4:1); t_R 13.68 min (70% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 236.67 [MH-Tr], 243.09 [Tr]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, 1H, *J* = 7.6 Hz), 7.50–7.43 (m, 2H), 7.39–728 (m, 10H), 7.03–6.94 (m, 10H), 2.32 (s, 3H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 164.18, 142.27, 141.25, 138.17, 136.34, 130.65, 129.21, 129.08, 128.58, 128.16, 127.92, 127.57, 127.24, 126.43, 82.84, 21.16 ppm.

4.2.5. N-(trityl)-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole (**7**)

Prepared according to reported procedure [9]. M.p. 136–138 °C; R_f 0.40 (hexanes:EtOAc, 4:1); t_R 13.07 min (70% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 315.41 [MH − Tr] (^{79}Br), 317.17 [MH − Tr + 2] (^{81}Br), 242.95 [Tr]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, 1H, *J* = 7.6 Hz), 7.66–7.58 (m, 2H), 7.53–7.33 (m, 10H), 7.28 (d, 2H, *J* = 8.0 Hz), 7.20 (d, 2H, *J* = 8.0 Hz), 7.04–7.02 (m, 6H), 4.53 (s 2H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 163.82, 141.11, 138.17, 130.62, 130.32, 130.21, 129.96, 129.61, 129.36, 128.48, 128.22, 127.91, 127.72, 82.96, 33.21 ppm.

4.2.6. 4′-methylbiphenyl-2-carboxylic acid (**8**)

Prepared according to reported procedure [9]. M.p. 139–140 °C; R_f 0.59 ($CHCl_3$:MeOH, 9.5:0.5); t_R 6.48 min (30% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 312.02 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, 1H, *J* = 7.6 Hz), 7.66–7.62 (m, 1H), 7.53–7.41 (m, 4H), 7.31 (d, 2H, *J* = 8.0 Hz), 2.43 (s, 3H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 171.49, 139.86, 137.79, 137.23, 134.25, 130.50, 130.45, 129.42, 129.08, 128.66, 127.38, 19.72 ppm.

4.2.7. Tert-butyl-4′-methylbiphenyl-2-carboxylate (**9**)

Prepared according to reported procedure [9]. R_f 0.41 (hexanes:EtOAc, 4:1); t_R 18.25 min (30% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 254.24 [MH − CH_3], 212.25 [MH − $C(CH_3)_3$]; ^{1}H NMR (400 MHz, CD_3OD): δ 7.69 (d, 1H, *J* = 7.6 Hz), 7.53 (t, 1H, *J* = 7.4 Hz), 7.41 (t, 1H, *J* = 7.4 Hz), 7.34 (d, 1H, *J* = 7.6 Hz), 7.23 (d, 2H, *J* = 8.0 Hz), 7.19 (d, 2H, *J* = 8.0 Hz), 2.41 (s, 3H), 1.29 (s, 9H) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 167.77, 140.67, 137.61, 135.64, 135.49, 131.83, 129.65, 129.51, 129.04, 129.01, 128.94, 127.83, 125.43, 80.19, 28.20, 17.17 ppm.

4.2.8. Tert-butyl-4′-(bromomethyl)biphenyl-2-carboxylate (**10**)

Prepared according to reported procedure [9]. R_f 0.39 (hexanes:EtOAc, 4:1); t_R 18.37 min (30% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 347.27 [MH] (^{79}Br), 349.29 [MH + 2] (^{81}Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, 1H, *J* = 7.6 Hz), 7.44–7.41 (m, 1H), 7.37–7.34 (m, 3H), 7.25–7.19 (m, 3H), 4.49 (s, 2H), 1.19 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 167.82, 142.16, 141.37, 136.61, 132.87, 130.73, 130.36, 129.61, 129.77, 129.01, 128.69, 127.34, 127.72, 81.44, 33.39, 27.57 ppm.

4.2.9. 4-butyl-1-[(2-(trimethylsilyl)ethoxy)methyl]imidazole (**12a**) and its regioisomer **12b**

To a solution of 4(5)-butylimidazole (**11**) (0.50 g, 4.03 mmol) in anhydrous DMF (15 mL) under argon atmosphere at 0 °C was added powdered dry NaH (95%, 0.12 g, 4.84 mmol) and the suspension was left at the same temperature for 15 min. Then, SEM-Cl (0.68 mL, 4.84 mmol) was added in three portions and the reaction mixture was allowed to warm to rt for 2 h. The reaction was quenched with 0.5 N NaOH (15 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (×2), dried (Na_2SO_4) and concentrated in vacuo. Purification by preparative RP-HPLC afforded **12a** (56%) as a yellow oil (ca. 2:1 **12a**/**12b** regioisomers by RP-HPLC and ^{1}H NMR spectroscopic analysis).

Alternatively, to a solution of 4(5)-butylimidazole (**11**) (1.50 g, 12.10 mmol) in anhydrous THF (30 mL) were added sequentially $Cyhex_2NMe$ (2.90 mL, 14.40 mmol) and SEM-Cl (1.72 mL, 9.68 mmol). After stirring the solution for 2 h, the reaction was quenched with 0.5 N NaOH (30 mL) and extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The crude oily residue was purified by preparative RP-HPLC (40% MeCN → 100% MeCN in 50 min) to afford **12a** (69%) as a yellow oil (ca. 3:1 **12a**/**12b** regioisomers by RP-HPLC). **12a**: R_f 0.53 ($CHCl_3$:MeOH, 9.5:0.5); t_R 16.24 min (5% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 255.08 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (s, 1H), 6.92 (s, 1H), 5.40 (s, 2H), 3.54 (t, 2H, *J* = 8.0 Hz), 2.71 (t, 2H, *J* = 7.4 Hz), 1.65 (quint, 2H, *J* = 7.4 Hz), 1.36 (sext, 2H, *J* = 7.4 Hz), 0.94–0.89 (m, 5H), −0.02 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 138.86, 136.17, 117.12, 79.51, 68.51, 31.69, 26.17, 23.50, 19.23, 15.06, 0.0 ppm. **12b**: Yellow oil. Yield 24%; R_f 0.53 ($CHCl_3$:MeOH, 9.5:0.5); t_R 16.58 min (5% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 255.14 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (s, 1H), 7.10 (s, 1H), 5.47 (s, 2H), 3.57 (t, 2H, *J* = 8.0 Hz), 2.69 (t, 2H, *J* = 7.5 Hz), 1.66 (quint, 2H, *J* = 7.5 Hz), 1.44 (sext, 2H, *J* = 7.5 Hz), 0.99–0.91 (m, 5H), 0.00 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 137.28, 132.20, 126.70, 73.78, 65.65, 30.28, 23.30, 22.29, 17.55, 13.74, −1.55 ppm.

4.2.10. 4-butyl-1-[(2-(trimethylsilyl)ethoxy)methyl]imidazole-2-carboxaldehyde (**13**)

To a solution of **12a** (0.50 g, 1.96 mmol) in anhydrous THF (10 mL) at −78 °C under argon was added *n*-BuLi (1.6 M in hexanes, 1.34 mL, 2.16 mL) dropwise via a syringe. The resulting yellow solution was

stirred at −78 °C for another 30 min and then anhydrous DMF (0.17 mL, 2.16 mmol) was added dropwise at the same temperature. The mixture was allowed to reach rt while being stirred for another 12 h. Then, the solution was quenched with saturated aqueous solution NH_4Cl (50 mL), the organic phase was extracted with EtOAc (3 × 50 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexanes:EtOAc, 1:4) to give the title compound (65%) as a yellow oil: R_f 0.58 (hexanes:EtOAc, 1:9); t_R 15.61 min (5% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 301.28 [MH + H_2O], 283.37 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H), 7.09 (s, 1H), 5.72 (s, 2H), 3.55 (t, 2H, *J* = 8.0 Hz), 2.84 (t, 2H, *J* = 7.5 Hz), 1.66 (quint, 2H, *J* = 7.5 Hz), 1.38 (sext, 2H, *J* = 7.5 Hz), 0.96–0.89 (m, 5H), −0.02 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 183.47, 147.88, 144.11, 123.79, 76.96, 68.26, 32.83, 29.45, 23.84, 19.28, 15.32, 0.00 ppm.

4.2.11. 4-butyl-2-hydroxymethyl-1-[(2-(trimethylsilyl)ethoxy) methyl]imidazole (**14**)

To a solution of **13** (0.40 g, 1.42 mmol) in MeOH (10 mL) at 0 °C was added $NaBH_4$ (70 mg, 1.85 mmol) portionwise over 20 min and stirred at 0 °C for an additional 1 h. The reaction mixture was then quenched with saturated aqueous solution NH_4Cl (40 mL), extracted with EtOAc (3 × 40 mL) and the organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Pure alcohol **14** (92%) was obtained as a yellow oil: R_f 0.57 ($CHCl_3$:MeOH, 9.5:0.5); t_R 15.90 min (5% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 285.39 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (s, 1H), 5.31 (s, 2H), 4.69 (s, 2H), 3.53 (t, 2H, *J* = 8.0 Hz), 2.51 (t, 2H, *J* = 7.5 Hz), 1.58 (quint, 2H, *J* = 7.5 Hz), 1.35 (sext, 2H, *J* = 7.5 Hz), 0.93–0.90 (m, 5H), 0.02 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 162.85, 147.06, 140.72, 127.90, 74.98, 66.47, 55.99, 31.20, 27.30, 22.32, 17.73, 13.87, 1.00 ppm.

4.2.12. 5-butyl-1-[[(2′-(2-trityl)-tetrazol-5-yl)biphenyl-4-yl] methyl]-3-[(2-(trimethylsilyl)ethoxy)methyl]imidazole (**15**)

To a stirred solution of **12a** (0.55 g, 2.16 mmol) in anhydrous MeCN (10 mL) under argon was added the alkylating agent **7** (1.32 g, 2.38 mmol) in one portion and the resulting mixture was heated at 70 °C for 3 h. Upon completion (disappearance of starting material confirmed by RP-HPLC), the solvent was concentrated, followed by recrystallization with Et_2O to afford **15** (78%) as a white powder: R_f 0.29 ($CHCl_3$:MeOH, 9.6:0.4); t_R 9.52 min (70% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 731.72 [M − Br]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.63 (s, 1H), 7.87 (d, 1H, *J* = 7.6 Hz), 7.55–7.14 (m, 17H), 6.92 (m, 6H), 5.53 (s, 2H), 5.42 (s, 2H), 3.65 (t, 2H, *J* = 8.0 Hz), 2.61 (t, 2H, *J* = 7.5 Hz), 1.59 (quint, 2H, *J* = 7.5 Hz), 1.38 (sext, 2H, *J* = 7.5 Hz), 0.98–0.89 (m, 5H), 0.03 (s, 9H) ppm.

4.2.13. 5-butyl-2-hydroxymethyl-1-[[(2′-(2-trityl)-tetrazol-5-yl) biphenyl-4-yl]methyl]-3-[(2-(trimethylsilyl)ethoxy)methyl] imidazole (**16**)

In a sealed tube were sequentially added **15** (1.20 g, 1.48 mmol), DMF (0.5 mL), 37% formalin (0.29 mL, 10.36 mmol) and diisopropylethylamine (0.65 mL, 3.70 mmol). The resulting mixture was stirred at 85 °C until HPLC showed no starting material left (ca. 1 h). Then, the mixture was quenched with 5% aqueous citric acid (10 mL), extracted with CH_2Cl_2 (3 × 20 mL) and the combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Recrystallization from Et_2O afforded **16** (89%) as a white powder.

Alternatively, compound **16** was prepared by direct alkylation of the alcohol **14** (1 eq) with **7** (1.1 eq) in MeCN at 70 °C for 12 h. Purification by flash column chromatography furnished the title compound: Yield 20%; R_f 0.25 ($CHCl_3$:MeOH, 9.6:0.4); t_R 8.31 min (70% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 761.65 [M − Br]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96–7.93 (m, 1H), 7.51–7.44 (m, 2H), 7.37–7.13 (m, 13H), 6.95–6.92 (m, 6H), 6.83 (d, 2H, *J* = 8.0 Hz), 5.90 (s, 2H), 5.42 (s, 2H), 4.70 (s, 2H), 3.70 (t, 2H, *J* = 8.0 Hz), 2.44 (t, 2H, *J* = 7.5 Hz), 1.55 (quint, 2H, *J* = 7.5 Hz), 1.31 (sext, 2H, *J* = 7.5 Hz), 0.96 (t, 2H, *J* = 8.0 Hz), 0.87 (t, 3H, *J* = 7.5 Hz), 0.01 (s, 9H) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 163.81, 146.47, 135.45, 131.79, 130.56, 130.25, 130.00, 128.36, 127.95, 127.88, 127.66, 126.18, 125.58, 117.26, 82.92, 78.24, 68.36, 51.97, 48.92, 28.76, 23.68, 22.06, 17.88, 13.58, 1.39 ppm.

4.2.14. 5-butyl-2-hydroxymethyl-1-[[(2′-(2-trityl)-tetrazol-5-yl) biphenyl-4-yl]methyl]imidazole (**17**)

Compound **16** (1.10 g, 1.31 mmol) was dissolved in a solution of 1 M TBAF in THF (6 mL) and the resulting mixture was heated to reflux under argon atmosphere for 8 h. Then, the solvent was concentrated and the residue was partitioned between CH_2Cl_2 (40 mL) and H_2O (30 mL). The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography ($CHCl_3$:MeOH, 9.7:0.3) afforded the alcohol **17** (90%) as a white foam: R_f 0.24 ($CHCl_3$:MeOH, 9.7:0.3); t_R 14.66 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 631.49 [MH], 243.33 [Tr]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (dd, 1H, *J* = 7.6, 1.4 Hz), 7.48–7.44 (m, 2H), 7.35–7.23 (m, 10H), 7.08 (d, 2H, *J* = 7.6 Hz), 6.93–6.71 (m, 9H), 5.07 (s, 2H), 4.46 (s, 2H), 2.29 (t, 2H, *J* = 7.5 Hz), 1.49 (quint, 2H, *J* = 7.5 Hz), 1.25 (sext, 2H, *J* = 7.5 Hz), 0.83 (t, 3H, *J* = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 163.94, 147.39, 135.11, 133.47, 130.73, 130.24, 129.75, 128.28, 127.64, 126.31, 125.27, 124.19, 82.89, 56.79, 46.35, 30.05, 23.98, 22.94, 13.79 ppm.

4.2.15. 5-butyl-2-hydroxymethyl-1-[[2′-(2H-tetrazol-5-yl) biphenyl-4-yl]methyl]imidazole (**21**)

Compound **17** (0.15 g, 0.24 mmol) was dissolved in a solution of 20% TFA in CH_2Cl_2 (2 mL) and then Et_3SiH (0.11 mL, 0.72 mmol) was added as a scavenger. The resulting solution was stirred at rt for 1 h before it was concentrated in vacuo. Precipitation from Et_2O furnished the TFA salt as an amorphous powder. The TFA salt was partitioned between H_2O (5 mL) and EtOAc (5 mL), followed by addition of $NaHCO_3$ (0.21 g, 2.5 mmol). Then, the mixture was adjusted to pH 3.0 with 1 N HCl, the organic phase was separated, dried (Na_2SO_4) and concentrated in vacuo to give **21** (85%) as a white solid: M.p. 109–111 °C; R_f 0.33 ($CHCl_3$:MeOH:AcOH, 8.5:1.5:0.5); t_R 8.52 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 389.13 [MH]; ^{1}H NMR (400 MHz, CD_3OD): δ 7.73–7.68 (m, 2H), 7.62–7.56 (m, 2H), 7.36 (s, 1H), 7.19 (d, 2H, *J* = 8.3 Hz), 7.13 (d, 2H, *J* = 8.3 Hz), 5.45 (s, 2H), 4.83 (s, 2H), 2.58 (t, 2H, *J* = 7.5 Hz), 1.58 (quint, 2H, *J* = 7.5 Hz), 1.39 (sext, 2H, *J* = 7.5 Hz), 0.93 (t, 3H, *J* = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 162.35, 149.26, 143.40, 143.27, 137.96, 135.50, 132.62, 132.04, 131.88, 131.41, 130.68, 129.41, 128.31, 128.03, 119.68, 56.97, 31.60, 25.36, 24.05, 14.88 ppm.

4.2.16. 5-butyl-4-chloro-2-hydroxymethyl-1-[[(2′-(2-trityl)-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole (**18**)

To a solution of **17** (0.30 g, 0.47 mmol) in anhydrous DMF (4 mL) was added NCS (70 mg, 0.52 mmol) in one portion. After stirring at 70 °C for 8 h, the reaction mixture was quenched with H_2O (2 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The oily residue was purified by flash column chromatography ($CHCl_3$) to afford the title compound **18** (86%) as a white foam: R_f 0.65 ($CHCl_3$:MeOH, 9.6:0.4); t_R 19.11 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (*m*/*z*): 665.46 [MH] (^{35}Cl), 667.48 [MH + 2] (^{37}Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d,

1H, J = 7.6 Hz), 7.51–7.44 (m, 3H), 7.36–7.23 (m, 8H), 7.10 (d, 2H, J = 8.0 Hz), 6.93–6.91 (m, 7H), 6.72 (d, 2H, J = 8.0 Hz), 5.07 (s, 2H), 4.24 (s, 2H), 2.36 (t, 2H, J = 7.5 Hz), 1.37–1.19 (m, 4H), 0.82 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 163.94, 146.28, 134.77, 131.10, 130.58, 130.35, 130.24, 128.69, 128.02, 126.58, 125.86, 125.62, 83.24, 57.21, 47.37, 31.22, 22.53, 22.94, 14.09 ppm.

4.2.17. 4-bromo-5-butyl-2-hydroxymethyl-1-[[(2′-(2-trityl)-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole (**19**)

To a solution of **17** (0.25 g, 0.40 mmol) in anhydrous DMF (4 mL) was added NBS (78 mg, 0.44 mmol) in one portion. After stirring at rt for 30 min the reaction mixture was quenched with H_2O (2 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The oily residue was purified by flash column chromatography ($CHCl_3$) to afford the title compound **19** (92%) as a white foam: R_f 0.64 ($CHCl_3$:MeOH, 9.6:0.4); t_R 18.54 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 709.50 [MH] (^{79}Br), 711.51 [MH + 2] (^{81}Br); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, 1H, J = 7.6 Hz), 7.49–7.44 (m, 3H), 7.36–7.24 (m, 8H), 7.10 (d, 2H, J = 7.6 Hz), 6.94–6.92 (m, 7H), 6.74 (d, 2H, J = 8.0 Hz), 5.09 (s, 2H), 4.43 (s, 2H), 2.36 (t, 2H, J = 7.5 Hz), 1.37–1.21 (m, 4H), 0.83 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 167.52, 147.28, 135.27, 132.10, 130.69, 130.25, 130.13, 129.48, 128.52, 126.58, 125.72, 125.26, 111.39, 83.55, 56.11, 47.15, 30.16, 22,56, 22.47, 15.52 ppm.

4.2.18. 5-butyl-2-hydroxymethyl-4-iodo-1-[[(2′-(2-trityl)-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole (**20**)

Compound **17** was prepared in an analogous manner to that described for **18** using NIS (1.5 eq). Yield 90%. R_f 0.64 ($CHCl_3$:MeOH, 9.6:0.4); t_R 16.50 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 757.71 [MH], 631.49 [MH − I]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (dd, 1H, J = 7.3 Hz, 1.7 Hz), 7.51–7.44 (m, 3H), 7.36–7.24 (m, 8H), 7.11 (d, 2H, J = 8.0 Hz), 6.93–6.91 (m, 7H), 6.74 (d, 2H, J = 8.0 Hz), 5.15 (s, 2H), 4.45 (s, 2H), 2.37 (t, 2H, J = 7.5 Hz), 1.36–1.21 (m, 4H), 0.84 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 164.25, 149.41, 141.61, 134.70, 130.57, 130.27, 128.70, 128.29, 128.02, 127.62, 125.64, 83.25, 81.70, 57.30, 47.86, 31.65, 24.97, 22.68, 14.13 ppm.

4.2.19. 5-butyl-4-chloro-2-hydroxymethyl-1-[[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole (**22**)

Compound **22** was prepared in an analogous manner to that described for **21**. Yield 82%; M.p. 105–107 °C; R_f 0.51 ($CHCl_3$:MeOH:AcOH, 90:15:5); t_R 10.73 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 423.41 [MH] (^{35}Cl), 425.87 [MH + 2] (^{37}Cl); ^{1}H NMR (400 MHz, CD_3OD): δ 7.71–7.67 (m, 2H), 7.57 (m, 2H), 7.14 (d, 2H, J = 8.0 Hz), 7.06 (d, 2H, J = 8.0 Hz), 5.35 (s, 2H, H-11), 4.55 (s, 2H), 2.49 (t, 2H, J = 7.5 Hz), 1.35–1.27 (m, 4H), 0.86 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 157.81, 145.28, 141.46, 139.38, 135.77, 130.27, 130.18, 129.50, 129.27, 129.23, 128.08, 125.91, 124.43, 55.70, 30.27, 22.19, 21.73, 12.60 ppm.

4.2.20. 4-bromo-5-butyl-2-hydroxymethyl-1-[[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole (**23**)

Compound **23** was prepared in an analogous manner to that described for **21**. Yield 79%; M.p. 103–105 °C; R_f 0.46 ($CHCl_3$:MeOH:AcOH, 9:1.5:0.5); t_R 10.55 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 467.20 [MH] (^{79}Br), 469.16 [MH + 2] (^{81}Br); ^{1}H NMR (400 MHz, CD_3OD): δ 7.74–7.67 (m, 2H), 7.58 (m, 2H), 7.16 (d, 2H, J = 8.0 Hz), 7.08 (d, 2H, J = 8.0 Hz), 5.43 (s, 2H), 4.65 (s, 2H), 2.53 (t, 2H, J = 7.5 Hz), 1.32–1.25 (m, 4H), 0.87 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 155.76, 146.79, 141.50, 139.08, 136.02, 130.93, 130.23, 129.24, 127.58, 127.54, 126.03, 123.50, 111.45, 55.72, 30.12, 24.05, 21.87, 12.65 ppm.

4.2.21. 5-butyl-2-hydroxymethyl-4-iodo-1-[[2′-(2H-tetrazol-5-yl) biphenyl-4-yl]methyl]imidazole (**24**)

Compound **24** was prepared in an analogous manner to that described for **21**. Yield 80%; M.p. 101–102 °C; R_f 0.47 ($CHCl_3$:MeOH:AcOH, 90:15:5); t_R 9.94 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 515.28 [MH]; ^{1}H NMR (400 MHz, CD_3OD): δ 7.69–7.65 (m, 2H), 7.55 (m, 2H), 7.14 (d, 2H, J = 8.1 Hz), 7.07 (d, 2H, J = 8.1 Hz), 5.44 (s, 2H), 4.60 (s, 2H), 2.37 (t, 2H, J = 7.5 Hz), 1.32–1.25 (m, 4H), 0.84 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 154.73, 149.37, 141.49, 139.07, 136.17, 130.27, 130.22, 129.27, 127.57, 126.07, 123.25, 102.43, 55.61, 30.66, 24.03, 21.87, 12.65 ppm.

4.2.22. 4-butyl-1H-imidazole-2-carboxaldehyde (**25**)

To a solution of the aldehyde **13** (120 mg, 0.42 mmol) in EtOH (2 mL) was added 3 N HCl (0.5 mL) and the resulting mixture was heated at 60 °C. After being stirred for 3 h, the reaction mixture was cooled to rt, quenched with saturated aqueous solution of K_2CO_3 (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography ($CHCl_3$:MeOH, 9.6:0.4) afforded **25** (63%) as a white solid: M.p. 97–99 °C; R_f 0.46 ($CHCl_3$:MeOH, 9.5:0.5); t_R 6.45 min (5% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 153.66 [MH]; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H), 7.13 (s, 1H), 2.57 (t, 2H, J = 7.4 Hz), 1.58 (quint, 2H, J = 7.4 Hz), 1.29 (sext, 2H, J = 7.4 Hz), 0.88 (t, 2H, J = 7.4 Hz) ppm; ^{13}C NMR (160 MHz, DMSO-d_6): δ 184.94, 149.57, 145.46, 130.52, 35.14, 29.69, 26.08, 18.06 ppm.

4.2.23. 5-butyl-1-[[(2′-(2-trityl)-tetrazol-5-yl)biphenyl-4-yl] methyl]imidazole-2-carboxaldehyde (**27**)

To a solution of the aldehyde **25** (0.10 g, 0.66 mmol) in anhydrous DMF (10 mL) under argon atmosphere were added sequentially anhydrous K_2CO_3 (0.18 g, 1.32 mmol) and **7** (0.41 g, 0.73 mmol). The reaction mixture was stirred at rt for 18 h before being quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with H_2O (3 × 40 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (hexanes:EtOAc, 6:4) furnished the title compound **27** (28%) as white foam (ca. 2.5:1 **26**/**27** regioisomers by RP-HPLC analysis).

Alternatively, aldehyde **27** was prepared by Swern oxidation. To a solution of anhydrous dimethyl sulfoxide (85 μL, 1.20 mmol) in anhydrous CH_2Cl_2 (3 mL) at −78 °C was added oxalyl chloride (48 μL, 0.56 mmol) such that the temperature did not exceed −65 °C. After 15 min, a solution of the alcohol **17** (0.20 g, 0.31 mmol) in CH_2Cl_2 (1 mL) was added and the reaction mixture was stirred at the same temperature for 50 min. Then, anhydrous Et_3N (160 μL, 1.24 mmol) was added and the reaction mixture was allowed to warm to rt for 1 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the organic phase was washed once with H_2O (20 mL), brine, dried (Na_2SO_4), filtered and concentrated. Purification by flash column chromatography (hexanes:EtOAc, 6:4) furnished the title compound **27** (92%) as a white foam. **26**: R_f 0.43 (hexanes:EtOAc, 3:7); t_R 14.24 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 629.48 [MH], 243.21 [Tr]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H), 7.92 (d, 1H, J = 7.6 Hz), 7.51–7.44 (m, 3H), 7.36–7.25 (m, 8H), 7.15 (s, 1H), 7.09 (d, 2H, J = 7.9 Hz), 6.94–6.92 (m, 7H), 6.80 (d, 2H, J = 7.9 Hz), 5.60 (s, 2H), 2.41 (t, 2H, J = 7.5 Hz), 1.54 (quint, 2H, J = 7.5 Hz), 1.29 (sext, 2H, J = 7.5 Hz), 0.87 (t, 2H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 181.57, 163.91, 146.87, 143.56, 141.44, 141.22, 140.75, 140.18, 134.52, 130.30, 130.74, 130.23, 129.94, 129.74, 128.25, 127.64, 126.27, 125.89, 82.89, 47.43, 27.70, 23.62, 22.31, 13.73 ppm. **27**: R_f 0.69 (hexanes:EtOAc, 3:7); t_R 13.75 min (40% MeCN → 100% MeCN in 30 min); ESI-MS (m/z):

629.54 [MH], 243.18 [Tr]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.73 (s, 1H), 7.90 (dd, 1H, J = 7.6 Hz, 1.2 Hz), 7.44–7.38 (m, 2H), 7.30–7.26 (m, 4H), 7.19–7.16 (m, 6H), 7.04 (d, 2H, J = 8.0 Hz), 6.88 (d, 2H, J = 8.0 Hz), 6.84–6.83 (m, 6H), 6.64 (s, 1H), 5.38 (s, 2H), 2.46 (t, 2H, J = 7.5 Hz), 1.51 (quint, 2H, J = 7.5 Hz), 1.29 (sext, 2H, J = 7.5 Hz), 0.84 (t, 2H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 181.91, 163.87, 146.30, 142.29, 141.39, 141.21, 134.51, 130.68, 130.21, 129.97, 129.79, 128.27, 127.62, 127.62, 127.19, 126.24, 123.15, 82.89, 50.24, 31.40, 28.05, 22.46, 13.87 ppm.

4.2.24. 5-butyl-1-[[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl] imidazole-2-carboxaldehyde (**29**)

To a solution of the aldehyde **27** (0.16 g, 0.25 mmol) in Et_2O (2 mL) was added formic acid (3 mL) and the resulting mixture was stirred at rt for 2 h. Then, the solvent was concentrated in vacuo, followed by recrystallization from diisopropyl ether to afford the title compound **29** (83%) as a white amorphous solid: R_f 0.45 ($CHCl_3$/MeOH/AcOH, 8.5:1.5:0.5); t_R 8.25 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 405.25 [MH + H_2O], 387.23 [MH]; ^{1}H NMR (400 MHz, CD_3OD): δ 9.68 (s, 1H), 7.57–7.51 (m, 2H), 7.47–7.45 (m, 2H), 7.17 (s, 1H), 7.09 (d, 2H, J = 8.0 Hz), 6.92 (d, 2H, J = 8.0 Hz), 5.68 (s, 2H), 2.57 (t, 2H, J = 7.5 Hz), 1.57 (quint, 2H, J = 7.5 Hz), 1.37 (sext, 2H, J = 7.5 Hz), 0.91 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 180.45, 157.68, 147.58, 141.24, 139.12, 135.23, 130.72, 130.30, 129.72, 129.67, 129.55, 128.49, 128.23, 127.57, 126.53, 126.06, 124.25, 55.72, 29.41, 23.11, 21.88, 12.62 ppm.

4.2.25. 5-butyl-1-[[2′-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl] imidazole-2-carboxylic acid (**30**)

To a solution of the aldehyde **27** (0.10 g, 0.16 mmol) in *t*-BuOH (1 mL) were sequentially added 2-methyl-2-butene (0.8 mL), NaH_2PO_4 (0.13 g, 1.10 mmol) and a solution of 80% $NaClO_2$ (0.19 g, 1.44 mmol) in H_2O (1 mL). The reaction mixture was stirred at rt for 1 h. The resulting solution was extracted with CH_2Cl_2 (3 × 20 mL) and the combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated to afford the carboxylic acid **28** (95%) as a white foam, which was used directly without further purification. Then, detritylation of **28** was accomplished by an analogous manner to that described for **29** to furnish **30** (86%).

Alternatively, the title compound **30** was prepared by oxidation with $KMnO_4$. To a solution of the alcohol **21** (80 mg, 0.21 mmol) in a mixture of H_2O/acetone 1:1 (2 mL) at 0 °C were sequentially added $KMnO_4$ (44 mg, 0.28 mmol) and a catalytic amount of 18-crown-6 (5 mg). The resulting mixture was allowed to warm to rt over a period of 1 h before it was quenched with MeOH (1 mL), followed by filtration through a pad of Celite®. The filtrate was adjusted to pH 4 with 0.1 M HCl, extracted with EtOAc (3 × 20 mL) and the combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Recrystallization from diisopropyl ether afforded the title compound **30** (74%) as an amorphous solid. **30**: R_f 0.21 ($CHCl_3$/MeOH/AcOH, 8.5:1.5:0.5); t_R 8.48 min (20% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 805.64 [2M + H], 403.30 [MH], 359.26 [MH-COOH]; ^{1}H NMR (400 MHz, CD_3OD): δ 7.68–7.67 (m, 2H), 7.58–7.54 (m, 2H), 7.29 (s, 1H), 7.15 (br s, 4H), 6.02 (s, 2H), 2.54 (t, 2H, J = 7.5 Hz), 1.55 (quint, 2H, J = 7.5 Hz), 1.38 (sext, 2H, J = 7.5 Hz), 0.91 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, CD_3OD): δ 172.10, 160.27, 146.60, 141.28, 139.42, 134.65, 130.75, 130.26, 129.32, 128.50, 128.22, 127.62, 126.58, 123.86, 51.52, 25.06, 24.05, 21.88, 14.85 ppm.

4.2.26. 5-butyl-1-[[2′-(tert-butoxycarbonyl)biphenyl-4-yl]methyl]-3-[(2-(trimethylsilyl)ethoxy)methyl]imidazole (**31**)

Compound **31** was prepared in an analogous manner to that described for **15**, using **10** as an alkylating agent. Yield 87%; R_f 0.31 ($CHCl_3$/MeOH, 9.6:0.4); t_R 8.79 min (60% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 521.58 [M − Br]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.95 (s, 1H), 7.77 (d, 1H, J = 7.6 Hz), 7.47–7.23 (m, 7H), 7.10 (s, 1H), 5.72 (s, 2H), 5.59 (s, 2H), 3.70 (t, 2H, J = 8.0 Hz), 2.54 (t, 2H, J = 7.5 Hz), 1.56 (quint, 2H, J = 7.5 Hz), 1.35–1.25 (m, 11H), 0.97–0.89 (m, 5H), 0.0 (s, 9H) ppm.

4.2.27. 5-butyl-1-[[2′-(tert-butoxycarbonyl)biphenyl-4-yl]methyl]-2-hydroxymethyl-3-[(2-(trimethylsilyl)ethoxy)methyl]imidazole (**32**)

Compound **32** was prepared in an analogous manner to that described for **16** by direct hydroxymethylation. Yield 93%; R_f 0.26 ($CHCl_3$/MeOH, 9.6:0.4); t_R 7.25 min (60% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 551.80 [M − Br]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 1H, J = 7.6 Hz), 7.49 (t, 1H, J = 7.4 Hz), 7.41 (t, 1H, J = 7.4 Hz), 7.32 (d, 2H, J = 8.0 Hz), 7.24 (s, 1H), 7.18 (m, 1H), 7.08 (d, 2H, J = 8.0 Hz), 5.78 (s, 2H), 5.67 (s, 2H), 4.87 (s, 2H), 3.68 (t, 2H, J = 8.0 Hz), 2.56 (t, 2H, J = 7.5 Hz), 1.62 (quint, 2H, J = 7.5 Hz), 1.56–1.25 (m, 11H), 0.96–0.88 (m, 5H), 0.0 (s, 9H) ppm.

4.2.28. 5-butyl-1-[(2′-carboxybiphenyl-4-yl)methyl]-2-hydroxymethylimidazole (**33**)

Compound **33** was prepared in an analogous manner to that described for **21**. Yield 82%; R_f 0.29 ($CHCl_3$/MeOH/AcOH, 8.5:1.5:0.5); t_R 12.43 min (10% MeCN → 100% MeCN in 30 min); ESI-MS (m/z): 365.17 [MH]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, 1H, J = 7.6 Hz), 7.50 (t, 1H, J = 7.4 Hz), 7.40 (t, 1H, J = 7.4 Hz), 7.32 (d, 2H, J = 7.6 Hz), 7.29 (s, 1H), 7.05-6.97 (m, 3H), 5.26 (s, 2H), 4.61 (s, 2H), 2.48 (t, 2H, J = 7.5 Hz), 1.53 (quint, 2H, J = 7.5 Hz), 1.34 (sext, 2H, J = 7.5 Hz), 0.87 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (160 MHz, $CDCl_3$): δ 172.82, 147.37, 142.55, 141.69, 135.49, 132.43, 131.55, 130.77, 130.55, 129.84, 127.97, 126.53, 125.35, 54.02, 48.07, 29.06, 23.88, 22.44, 13.94 ppm.

4.3. Pharmacological evaluation

4.3.1. AT1 receptor binding assay of synthesized ANG II analogues cell culture and transfection

Human embryonic kidney (HEK 293) cells were grown in DMEM/F12 (1:1) containing 3.15 g/L glucose and 10% bovine calf serum at 37 °C and 5% CO_2. 60 mm dishes of HEK 293 cells at 80–90% confluence were transfected with 3 μg of plasmid DNA encoding the human AT1 receptor, using 9 μL of Lipofectamine and 2 mL of OPTIMEM. To generate stably transfected pools of cells expressing the AT1 receptor 5–12 h after transfection, the medium was replaced by DMEM/F12 (1:1) containing 3.15 g/L glucose, 10% bovine calf serum and 700 μg/mL of the antibiotic, Geneticin. The antibiotic use ensured the selection of a stably transfected pool of cells.

4.3.2. Harvesting cells and membrane preparation

HEK 293 cells (grown in 100 mm dishes) stably expressing the human AT1 receptor were washed with phosphate-buffered saline (PBS; 4.3 mM $Na_2HPO_4{\cdot}7H_2O$, 1.4 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2–7.3 at rt), briefly treated with PBS containing 2 mM EDTA (PBS/EDTA), and then dissociated in PBS/EDTA. Cell suspension was centrifuged at 1000× *g* for 5 min at rt, and the pellet was homogenized in 1 mL of buffer O (50 mM Tris–HCl containing 0.5 mM EDTA, 10% sucrose, 10 mM $MgCl_2$, pH 7.4 at 4 °C) using a Janke & Kunkel IKA Ultra Turrax T25 homogenizer (at setting ~20, 10–15 s, 4 °C). The homogenate was centrifuged at 250× *g* for 5 min at rt. The pellet was discarded and the supernatant was centrifuged (16,000× *g*, 10 min, 4 °C). The membrane pellet was resuspended (0.6–0.7 mL/100 mm dish) in buffer B (50 mM Tris–HCl containing 1 mM EDTA, 10 mM $MgCl_2$, 0.2% BSA,

0.2 mg/mL bacitracin, and 0.93 μg/mL aprotinin, pH 7.4 at 4 °C) and used for radioligand binding studies.

4.3.3. [^{125}I–Sar^1–Ile^8] ANG II binding

The [^{125}I–Sar^1–Ile^8] ANG II competition binding was performed as follows. Aliquots of diluted membrane suspension (50 μL) were added into tubes, containing buffer B and 100,000–120,000 cpm [^{125}I–Sar^1–Ile^8] ANG II with or without increasing concentrations of ANG II analogues in a final volume of 0.15 mL. The mixtures were incubated (1 h, 24 °C) and then, filtered using a Brandel cell harvester through Whatman GF/C glass fiber filters, presoaked for 1 h in 0.5% polyethylenimine at 4 °C. The filters were washed 10 times with 1–2 mL of ice-cold 50 mM Tris–HCl containing NaCl 120 mM, pH 7.4 at 4 °C. Filters were assessed for radioactivity in a gamma counter (LKB Wallac 1275 minigamma, 80% efficiency). The amount of membrane used was adjusted to ensure that specific binding was always equal to or less than 10% of the total concentration of the radioligand added. Nonspecific binding of [^{125}I–Sar^1–Ile^8] ANG II binding was estimated in the presence of 1000 nM [Sar^1–Ile^8] ANG II. Specific binding was defined as total binding minus nonspecific binding [57]. Data analysis for competition binding was performed by nonlinear regression analysis, using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). −log IC_{50} values were obtained by fitting the data from competition studies to a one-site competition model and presented as mean plus/minus standard error (SE).

4.3.4. Rat uterotonic test in vitro

The test was performed in the same way as described for oxytocin and vasopressin [58–63] or bradykinin [64] analogues. The excised and longitudinally cut strips of rat uterus were placed into a bathing chamber into media without magnesium ions and hooked up to recorder of contractions. The height of the single isometric contraction of a uterine strip was measured. The cumulative dose–response curves of standard ANG II were constructed, i.e. doses of standard (in the presence or absence of analogues) were added successively to the uterus in the organ bath in doubling concentrations and at 1 min intervals without the fluid being changed until the maximal response (the highest contraction) was obtained. The shift of the curves in the presence of the compounds was determined. The concentration of the compounds leading to the shift corresponding to 0.3 in logarithmic scale (it means that twice higher concentration of the standard was necessary to reach the half maximal effect) was determined. Negative logarithm on the base of 10 of that concentration is pA_2. All samples were dissolved in DMSO to make stock solution of 1–4 mg/mL. Further dilutions were made in physiol. solution. Standard ANG II, oxytocin or bradykinin were dissolved in physiol. solution. Each analogue was tested using uteri from 3 to 5 different animals; the values in the Table 1 are averages ± SEM. Specificity of the effect was tested using construction of bradykinin and oxytocin dose–response curves in the presence of the analogues.

Wistar rats were used in all experiments. Handling of the experimental animals was done under supervision of the Ethics Committee of the Academy of Sciences according to § 23 of the law of the Czech Republic no. 246/1992.

4.4. Glide/Induced Fit Docking (IFD) studies

Geometry optimization calculations for studied ligands were performed with the Schrodinger's Maestro module using Polak–Ribiere conjugate gradient (PRCG) minimization (0.0001 kJ Å^{-1} mol^{-1}, convergence criteria) [65,66]. Protonation states of ligands and residues were tested using LigPrep and Protein Preparation modules under Schrodinger package at neutral pH. The receptor was mapped with grid-based calculations. In grid-based calculation procedure the target protein is embedded in a three-dimensional grid. Then, a probe atom is sequentially located at each grid point, the interaction energy between the probe atom and the target atoms is computed, and the value is stored in the grid. The energy of interaction of this single atom with the protein is assigned to the grid point. The active site was defined by 20 and 46 Å inner and outer cubic grid boxes, centered on the point that is the center of mass of residues K199 and H256. The Glide XP (extra precision) (v5.0) [66] combined with Induced Fit Docking (IFD) have been used for the docking calculations. IFD uses the Glide ligand docking program to account the ligand flexibility, and the Refinement module and the Prime algorithm to account for flexibility of the receptor. Schrodinger's IFD protocol model uses the following steps (the description below is from the IFD user manual): (i) constrained minimization of the receptor with an RMSD cut-off of 0.18 Å; (ii) initial Glide docking of each ligand using a soft potentials (0.5 van der Waals radii scaling of non-polar atoms of ligands and receptor using partial charge cut-off of 0.15); (iii) derived docking poses were refined using the Prime module of the Schrodinger. Residues within the 5.0 Å of ligand poses were minimized in order to form suitable conformations of poses at the active site of the receptor; (iv) Glide re-docking of each protein–ligand complex.

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