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New losartan-hydrocaffeic acid hybrids as antihypertensive-antioxidant dual drugs: Ester, amide and amine linkers

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ABSTRACT

We report new examples of a series of losartan–hydrocaffeic hybrids that bear novel ester, amide and amine linkers. These compounds were made by linking hydrocaffeic acid to the side chain of losartan at the C-5 position of the imidazole ring through different strategies. Experiments performed in cultured cells demonstrate that these new hybrids retain the ability to block the angiotensin II effect and have increased antioxidant ability. Most of them reduced arterial pressure in rats better or as much as losartan.

1. Introduction

Drug hybridization strategies are valuable tools in the discovery of new drugs with either improved affinity for one bioreceptor or dual action on more than one bioreceptor related with a multifactorial disease [1,2]. The hybridization approach has been applied, for instance, to combat drug-resistant bacteria [3,4] or parasites [5] and in the development of hybrid anticancer [6] or cardiovascular [7,8] drugs.

Antihypertensive drugs exert their main protective mechanism through lowering blood pressure, although the beneficial effects of these drugs can also be explained in conjunction with other possibilities. A relevant mechanism of cardiovascular disease progression in hypertensive patients may also be increased oxidative stress at the vascular level. Thus, treatments that interfere with this oxidative stress could be useful tools for management of these individuals.

Angiotensin converting enzyme (ACE) inhibitors have been widely used in hypertension therapy. However, in the past two decades, ACE inhibitors have been partially displaced by antagonists of angiotensin II, mainly because the latter are free of the side effects (mainly coughing) associated with ACE inhibitors. The angiotensin II antagonists (sartans), of which losartan (Fig. 1) [9] is the first marketed drug of this type, exert their antihypertensive effect by blocking the angiotensin II AT1 receptor [10].

Lowering blood pressure is not the only beneficial effect of angiotensin II blockade-based antihypertensives in the prevention of cardiovascular morbidity and mortality. Angiotensin II plays a significant role in the progression of tissue damage in cardiovascular diseases [11]. In this context, some chemical interventions have been performed on sartans in order to increase their ability to prevent tissue damage in the cardiovascular system while retaining their antihypertensive potency [12].

It is well established that oxidative stress is a mechanism that leads to the development of vascular damage [13]. The harmful effects of the increased local synthesis of reactive oxygen species is a consequence, at least partially, of different pathogenic stimuli involved in cardiovascular diseases, such as activated macrophages, hyperglycaemia, oxidized low density lipoprotein (LDL), and even

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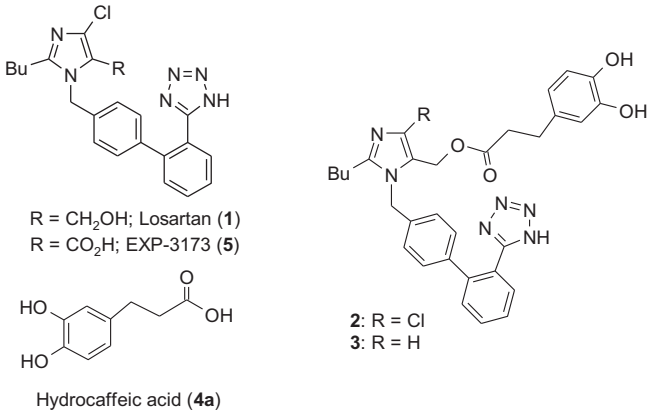


Fig. 1. Dual antihypertensive-antioxidant hybrids **2** and **3**.

angiotensin II [14–17]. These oxygen-active metabolites induce significant endothelial dysfunction [18–20] and are able to modify the normal balance between proliferation, apoptosis, and extracellular matrix synthesis in heart and arterial walls [21–23]. Some beneficial effects of antihypertensive molecules have been attributed, at least in part, to their antioxidant ability [24–26].

We recently reported a hybridization approach to dual drugs by linking the antihypertensive losartan (**1**) to antioxidant phenols [27]. The novel losartan-antioxidant hybrids **2** and **3** (Fig. 1), which were made by adding hydrocaffeic acid (**4a**) to the hydroxymethyl side chain of **1**, retain the ability to block the angiotensin II effect and have increased antioxidant ability. With this strategy, we tried to block the angiotensin II receptor and to deliver significant amounts of antioxidant molecules to the vascular walls, simultaneously. In hypertensive rats, compound **2** showed properties that suggest it may be more useful than **1** for controlling hypertension and cardiovascular damage. In addition, it prevented oxidative vascular damage more effectively than an equimolar mixture of **1** and the antioxidant fragment **4a.** These beneficial effects were due to the presence of significant amounts of compound **2** in plasma (90.3–93.5% after oral administration), since it was stable to plasmatic hydrolases after a 4-week treatment.

Following on from our studies on the role of the linker on the activity, herein we report different strategies for the incorporation of new ester, amide and amine linkers between the losartan and the antioxidant moiety to generate new hybrids. These new losartan analogues have been evaluated by their ability to block the angiotensin II effect and their antioxidant and antihypertensive activities.

2. Results and discussion

2.1. Antioxidant activity of phenols

The antioxidant ability of an array of 8 phenols was performed using the ABTS method and compared with losartan as we reported previously (Fig. 2) [27]. This is an electron transfer-based antioxidant capacity assay [28] first reported by Miller and Rice-Evans [29] and later improved [30]. The principle of this antioxidant assay is the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide. The ferryl myoglobin radical can oxidize 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) to generate a radical anion $ABTS^{\bullet-}$ (since sulfonic acid groups are fully deprotonated), a soluble chromogen that is blue/green in colour and can be determined spectrophotometrically at 600 nm. Antioxidant suppress this reaction by electron donation radical scavenging and inhibit the formation of the coloured radical anion $ABTS^{\bullet-}$ in a concentration dependent manner and the colour

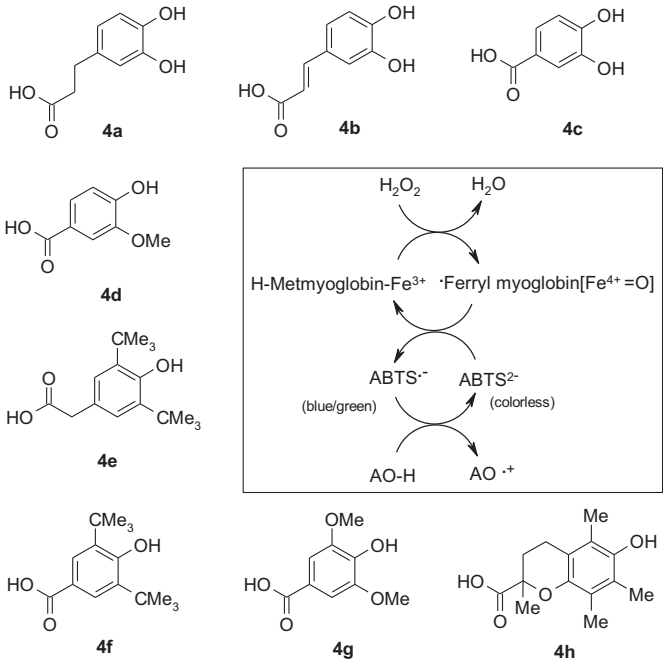


Fig. 2. Metmyoglobin/ABTS antioxidant assay (AO-H = antioxidant) and structures of phenols tested.

intensity decreases proportionally. The antioxidant ability is expressed as TEAC (Trolox Equivalent Antioxidant Capacity), that is, the antioxidant equivalent concentration in mM that gives the same percentage change of absorbance of the $ABTS^{\bullet-}$ as that of 1 mM of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, **4h**). Antioxidant capacity may also be expressed as ABTS inhibition percentage [31].

A heriarchical antioxidant potency order could be established for the phenols tested (Table 1): **4a** > **4g** ≈ **4e** ≈ **4h** (reference) > **4b** ≈ **4d** > **4f** ≈ **4c** ≈ **1**. Antioxidant capacity of some of these compounds was previously reported using a method slightly different [32]. Antioxidant capacity test showed that the best phenols were hydrocaffeic acid (**4a**), syringic acid (**4g**), and 3,5-di-*tert*-butyl-4-hydroxyphenyl acetic acid (**4e**). Phenols tested were 6–7-fold more potent than losartan (**1**), being **4a** the most potent antioxidant phenol assayed. Then, **4a** was selected to be linked to losartan through different linkers and was compared to **1** and our previously reported hybrid **2**. For each of the hybrids the number of atoms in the spacer between the phenolic and imidazole rings was the same than that of **2**.

2.1.1. Structure–activity relationship (SAR) studies

Some correlations are evident between the antioxidant activity measured and the type of spacer linking the aromatic ring to the carboxylic acid group or the type of substituent on *ortho*-position to

Table 1
Antioxidant capacity assay for phenols **4a**-**g** and losartan.

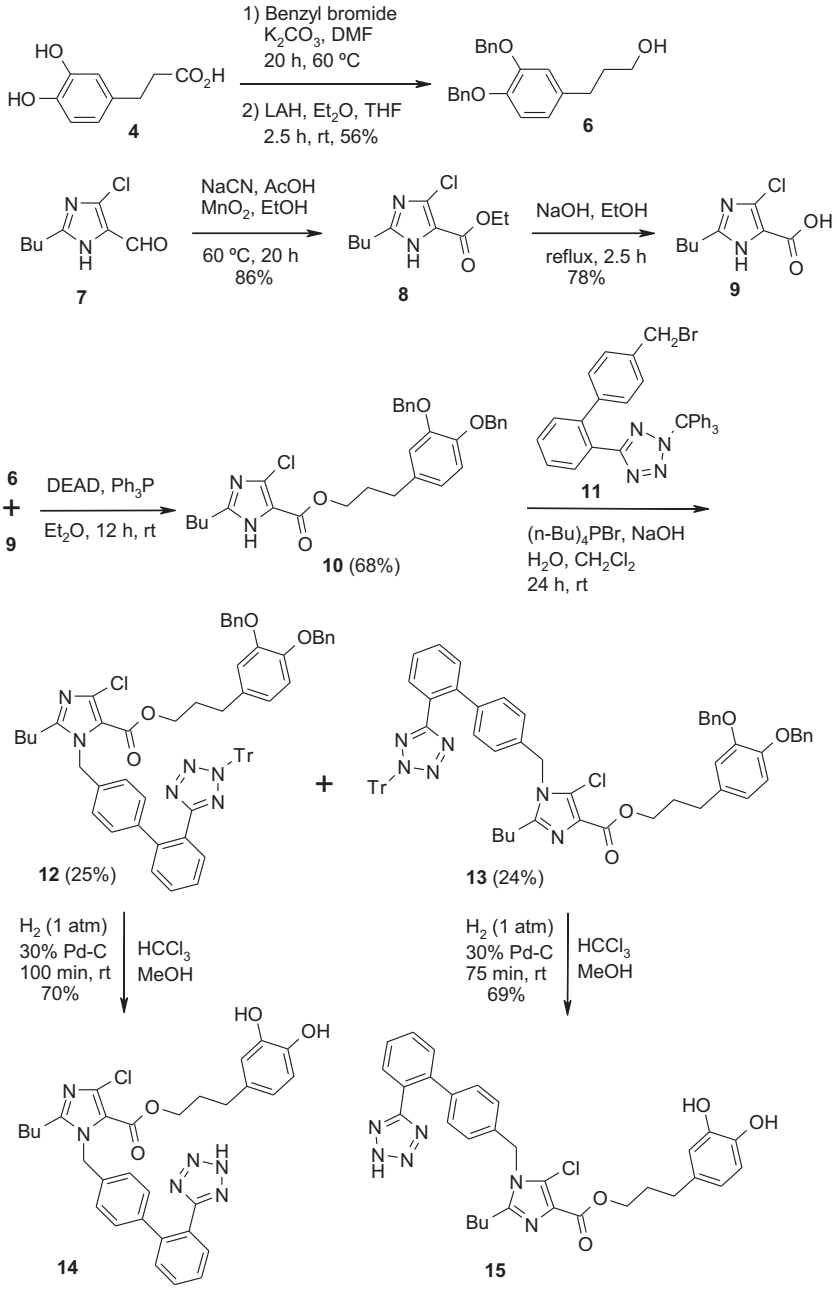
Compound	TEAC (mM, $n = 3$)[a]
4a	1.274 ± 0.069
4b	0.200 ± 0.099
4c	0.153 ± 0.085
4d	0.202 ± 0.014
4e	1.058 ± 0.115
4f	0.181 ± 0.059
4g	1.067 ± 0.141
1	0.179 ± 0.013

[a] Trolox (**4h**) as reference standard (see Experimental).

the hydroxyl group responsible for the activity (on the *para*-position to the spacer). The presence of a methylene group linked to the phenol ring seems to enhance the radical scavenging properties against $ABST^{\cdot-}$ (**4a** *vs* **4b** and **4c**; **4e** *vs* **4f**). Also, a double-bond resulted more active than if there is not a spacer (**4b** *vs* **4c**). Regarding to the type of substituent, a methoxy group is slightly better than a hydroxyl group (**4d** *vs* **4c**) and much better than a *t*-butyl group (**4g** *vs* **4f**).

2.2. Chemistry

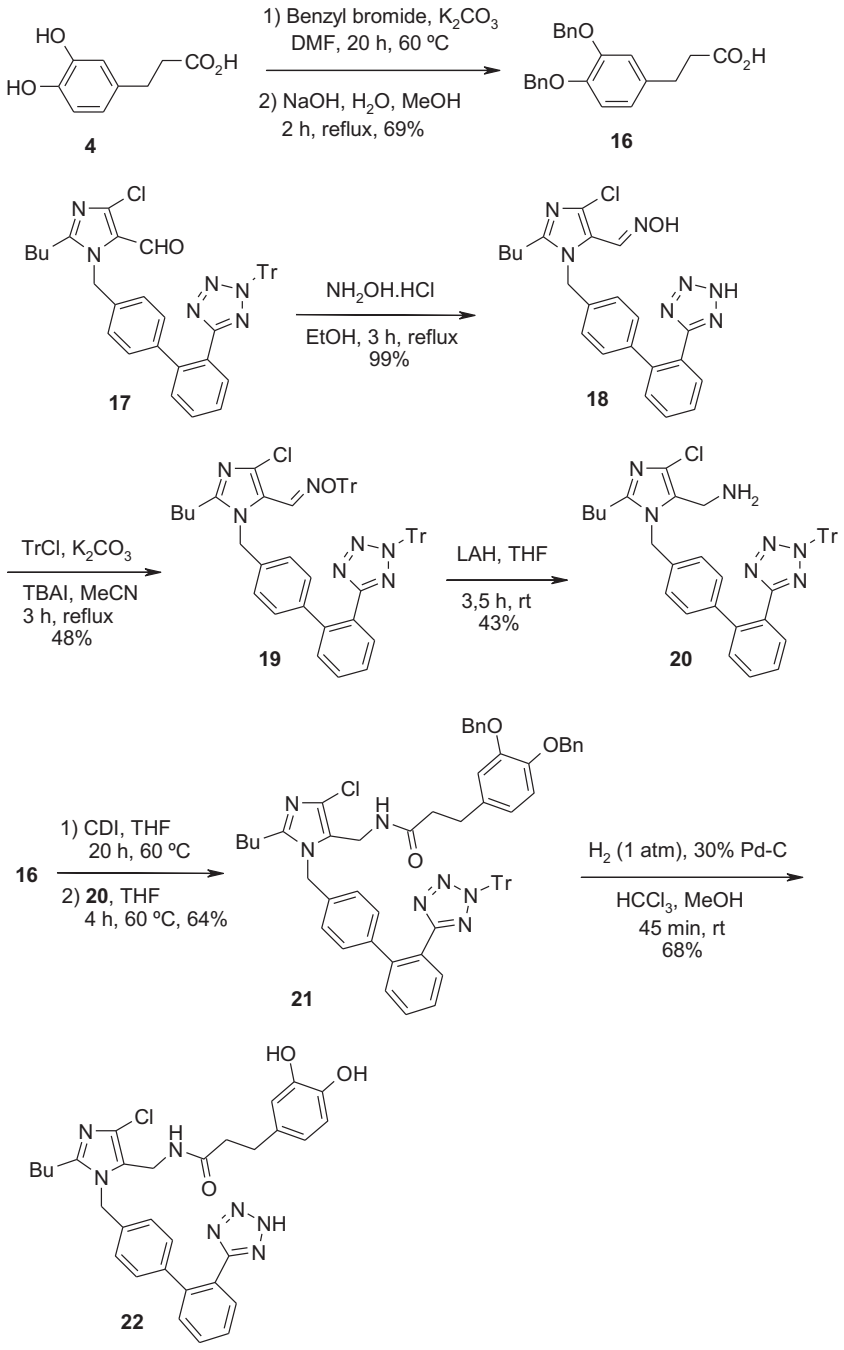
We first planned to prepare a new hybrid bearing an ester linker between losartan and the antioxidant moieties but with the reverse position of these fragments around the ester group in **2** (Scheme 1). Thus, hydrocaffeic acid (**4a**) was treated with excess benzyl bromide and the product was reduced with LAH to give alcohol **6** (56%). On the other hand, aldehyde **7** was converted to ester **8** (86%) in a one-pot three-step process involving cyanohydrin formation, oxidation and alcoholysis [33]. Ester **8** was then hydrolyzed in a basic medium to give acid **9** (78%). Alcohol **6** and acid **9** were linked in a Mitsunobu reaction to yield ester **10** (68%) [34]. Compound **10** was alkylated under phase-transfer conditions with bromide **11**, which was prepared as reported previously [9]. An equimolar regioisomeric mixture of esters **12** (25%) and **13** (24%) was obtained and separated by chromatography. Both **12** and **13** were hydrogenated at atmospheric pressure to remove trityl and benzyl groups to give hybrids **14** (70%) and **15** (69%), respectively. It was observed that the hydrogenation time is dependent on the batch of catalyst used. It was critical to monitor the reaction by TLC in order to minimize reduction of the C–Cl bond.



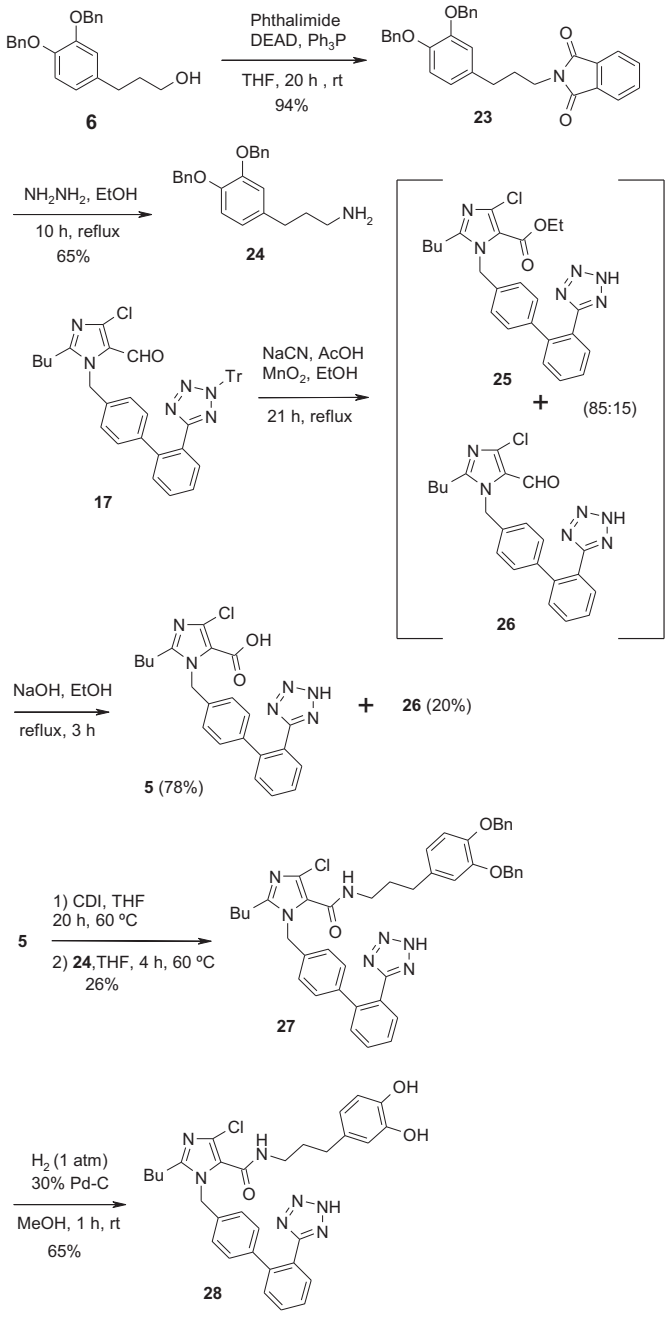
Scheme 1. Synthesis of esters **14** and **15**.

A hybrid bearing an amide linker was prepared next. In this case the losartan moiety bears the amine functional group and the antioxidant moiety bears the carboxylic acid group (Scheme 2). Hydrocaffeic acid (**4a**) was protected as the dibenzyl ether **16** as reported previously [27]. Reaction of **4a** with excess benzyl bromide and subsequent basic hydrolysis gave carboxylic acid **16** (69%). Aldehyde **17** was prepared as previously reported [9] and was then reacted with hydroxylamine to give oxime **18** quantitatively with simultaneous deprotection of tetrazole ring. The reduction of **18** was tested with LAH in Et_2O or THF, but the reaction did not take place. We then protected **18** with trityl chloride since oxime **18** proved to be highly insoluble in both polar and non polar solvents. Reaction of **18** with excess trityl chloride in the presence of TBAI yielded the fully protected compound **19** (48%). Subsequent reduction of the *O*-trityl-oxime **19** was performed with LAH to give amine **20** in modest yield (43%). The amidation reaction between **16** and **20** was carried out using CDI to yield amide **21** (64%). Finally, complete removal of the benzyl and trityl protecting groups was carried out by catalytic hydrogenation to give the hybrid **22** (72%).

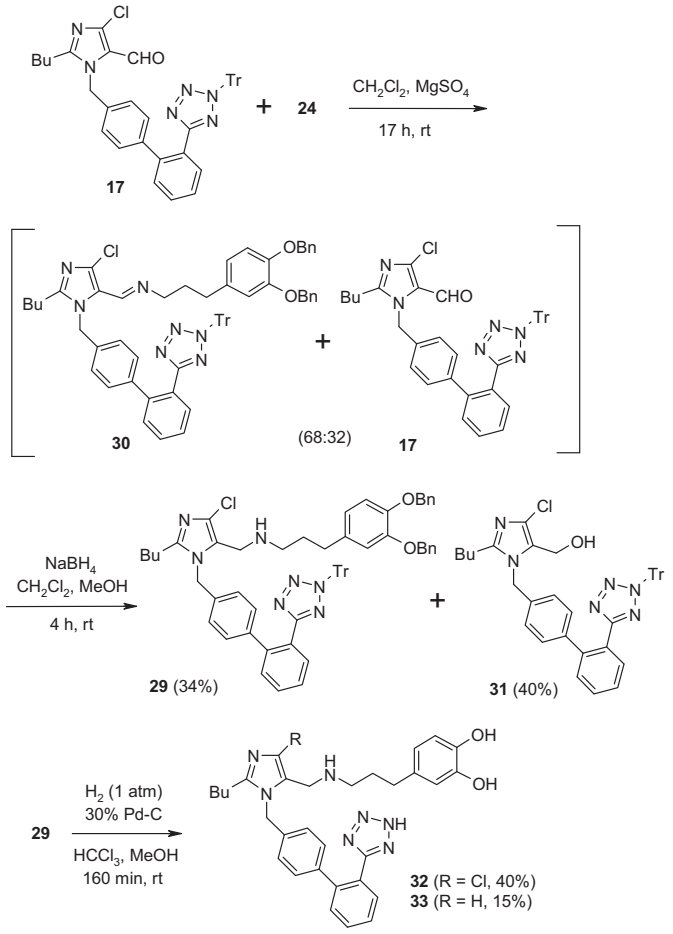
We also explored the synthesis of an amide hybrid bearing both losartan and antioxidant moieties in reverse positions (Scheme 3). Firstly, alkylation of phthalimide with alcohol **6** was performed under Mitsunobu conditions to give phthalimide **23** (94%), which was then reacted with hydrazine to yield amine **24** (65%). Aldehyde **17** was used as the starting material for the preparation of the carboxylic acid **5** (EXP-1371), the active metabolite of losartan [35].



Scheme 2. Synthesis of amide **22**.



Scheme 3. Synthesis of amide **28**.



Scheme 4. Synthesis of amine **32**.

Aldehyde **17** was treated under similar conditions as for **7** to give an inseparable mixture of ester **25** and aldehyde **26** (85:15). The isolated mixture was then hydrolyzed under basic conditons to give acid **5** (78%) and aldehyde **26** [9] (20%) which were then separated. Subsequent amidation between **5** and **24** was carried out using similar conditions as for **16** to yield amide **27** in low yield (26%), probably as a consequence of the lower reactivity of the imidazole-carboxylic acid **5**. Finally, the protecting groups of **27** were removed by catalytic hydrogenation to give the hybrid **28** (65%).

Another linker of interest was the amino group (Scheme 4). The link between the losartan and antioxidant units was formed by reductive amination. Reaction of amine **24** and aldehyde **17**, followed by addition of sodium borohydride gave amine **29**. Imine formation was not complete after 17 h and resulted in a mixture of **30**:**17** (68:32) as determined by ^{1}H NMR analysis. This mixture was further reacted with sodium borohydride to give amine **29** (34%) and trityl-protected losartan **31** (40%) [9]. Amine **29** was subsequently deprotected by catalytic hydrogenation for 2,5 h to give the amine **32** (40%) and dehalogenated product **33** (15%). In order to avoid reduction of C–Cl bond, the process was carried out in shorter times but conversion of starting material was not complete and yield could not be improved.

2.3. Spectroscopic data

^{1}H and ^{13}C NMR spectra were recorded for the synthesized hybrids using standard experiments in CD_3OD. Data were collected and compared between either the hybrids or between them and losartan (see Fig. 3). As expected no significant differences were found for chemical shifts of the protons and carbons at the butyl chain. The mean values for δ_{H1} (0.90 ± 0.02 ppm), δ_{H2} (1.35 ± 0.04 ppm), δ_{H3} (1.59 ± 0.03 ppm) and δ_{H4} (2.54 ± 0.10 ppm) were calculated. These values match with the chemical shifts of the corresponding protons of losartan (0.89, 1.33, 1.56, 2.59 ppm respectively). For δ_{H5}, slightly higher but still not significant deviations were found for ester **14** (5.59 ppm) and amide **28** (5.46 ppm) in comparison with losartan (5.30 ppm). These protons are shifted to lower field probably due to both electronic and anisotropic effects of the carbonyl group in position C-6. For hybrids **2** (5.08 ppm), **15** (5.30 ppm), **22** (5.02 ppm) and **32** (5.27 ppm), δ_{H5} is slightly lower or the same that than of losartan (5.30 ppm). For hybrids **2**, **22** and **32**, δ_{H6} (5.00, 4.28 and 3.73 ppm) correlates with the presence of carbonyloxy, carbonylamino and amino groups

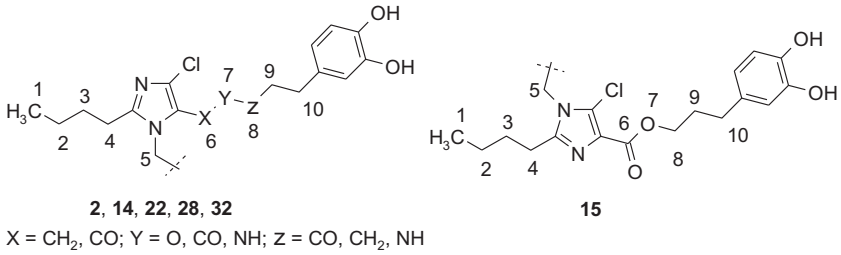


Fig. 3. General structures for NMR data.

respectively and with the hydroxy group in losartan (4.50 ppm). For protons at C8–C10 positions δ match with calculated values. In the ^{13}C NMR spectra there are not significant differences between hybrids and losartan for δ_{C4} ($\Delta\delta = 0.4$ ppm) and δ_{C5} ($\Delta\delta = 1.5$ ppm). ^{13}C NMR data showed that the carbonyl group of both ester **2** ($\delta_{CO} = 175.1$ ppm) and amide **22** ($\delta_{CO} = 174.9$ ppm) correlates with an alkanoate ester and an alkanamide respectively. For **14** ($\delta_{CO} = 162.0$ ppm), **15** ($\delta_{CO} = 163.3$ ppm) and **28** ($\delta_{CO} = 162.2$ ppm) the carbonyl group correlates with a conjugated ester or amide. According to proton and carbon chemical shift values for hybrids and losartan, the small differences observed do not suggest conformational preferences other than that of losartan and for this reason NOE experiments were not performed. As reported previously by Mavromoustakos [36,37] and Yoo [38] for losartan and other non peptide angiotensin II AT_1 antagonists, protons at C-4 and C-5 positions are pointing out each other. The slight downfield shift observed for H_5 in **14** and **28** supports this idea. This downfield shift should be stronger if protons at C-5 position were pointing out towards the carbonyl group at C-6. Hybrids **2**, **15**, **22** and **32**, which lack of this group at C-6, show the same δ_{H5} value than losartan or slightly shifted to upper field. Thus, we can conclude that, in methanol, these hybrids keep a conformation close to that reported for losartan.

2.4. Biological activities

2.4.1. Antioxidant activity of hybrids

The antioxidant properties of the different compounds (Fig. 4) was evaluated by the same method used for the phenols **4** and our previous reported ester hybrids (*Total antioxidant status assay kit*, Calbiochem, La Jolla, CA, U.S.A) [29]. Losartan (**1**) or its metabolite **5** displayed minimal antioxidant ability (0.03 ± 0.01 mM) and this increased 3–9 fold in the synthesized compounds, with **15** and **22** showing the best antioxidant ability (Table 2) [39–41]. Antioxidant ability is expressed as antioxidant equivalent concentration, in mM per 1 mM of product. The results are the mean of 8 independent experiments and SEM are in the range between ±0.01 and ±0.04 with $p < 0.05$ vs **1**. All the new hybrids elicited lower antioxidant ability than ester hybrid **2** (0.31 ± 0.03 mM). At concentrations of 1 mM, this product displays the same antioxidant ability as 1 mM of compound **4a** (0.36 ± 0.03 mM).

Table 2
Antioxidant ability and inhibition of cellular contraction of the new hybrids prepared and reference compounds.

Compound	TEAC (mM; $n = 8$)	Cell contraction inhibition (%; $n = 5$)
1[a]	0.03 ± 0.01	93
2	0.31 ± 0.03	94
4a	0.36 ± 0.03	85
5[b]	0.03 ± 0.01	–
14	0.10 ± 0.02	88
15	0.24 ± 0.03	95
22	0.27 ± 0.04	90
28	0.17 ± 0.02	93
32	0.13 ± 0.02	92
Ang II	–	82

[a] Losartan.
[b] 2-Butyl-5-chloro-3-[2′-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid (EXP-3147). It is the major active metabolite of losartan in the liver, and primarily responsible for the therapeutic response (see Ref. [39]). EXP3174 is 10–40 fold more potent than **1** and has a longer duration of action (see Ref. [40] and [41]).

2.4.1.1. Structure–activity relationship (SAR) studies. A hierarchical antioxidant potency order could be established for the hybrids prepared and it was compared to hydrocaffeic acid (**4a**), losartan (**1**) and its metabolite **5**: **4a** > **2** > **22** ≈ **15** > **28** > **32** > **14** > **1** ≈ **5**. Then, to obtain a good antioxidant activity, losartan must be the alcohol or amine moiety of both ester (**2** *vs* **14**) and amide (**22** *vs* **28**) hybrids. However, there is not a strong preference between an ester or amide for the antioxidant activity (**2** *vs* **22** and **14** *vs* **28**). Hybrid **15**, where losartan is the carboxylic acid moiety of the ester linker, also gives a clear antioxidant activity. This means that a reverse ester group is better accepted when the bifenilmethyl residue is linked to the nitrogen *N*-1 atom of the imidazole ring (**15** *vs* **14**). The compound **15** is an exception by its structure, among all the series indicated, and should be further explored in future papers.

2.4.2. Analysis of the ability to block angiotensin II-dependent cell contraction

The ability of angiotensin II to reduce the planar cell surface area (PCSA) in cells pre-treated with the different compounds was analyzed. Changes in PCSA are considered to be a consequence of cell contraction, and are due to the interaction of angiotensin II with its receptor [42]. The analysis was performed in cultured smooth muscle cells, incubated with angiotensin II, in the presence of **1** or the different synthesized compounds, for 30 min (the concentration of angiotensin II and the different compounds were 1 μM). Photographs (TMS-F Photomicroscope, Nikon, Tokyo, Japan, magnification 150×) of the same cells were taken at times 0 and 30 min. PCSA was determined by computer-aided planimetric techniques.

Results are included in the Table 2. The different compounds tested inhibited the angiotensin II-induced PCSA reduction. This inhibition ranged between 95% with **15** and 88% with **14**. However, statistically significant differences were not observed in the inhibition of the contraction shown by the different newly synthesized compounds, with respect to **1** and **2**, except for compound **14**. These results show that the attachment of hydrocaffeic acid (**4a**) to **1** is able to increase the antioxidant ability without markedly modifying its basic properties as an angiotensin II receptor blocker.

2.4.3. Antihypertensive activity

Wistar rats were treated for 1 week with an inhibitor of the synthesis of nitric oxide, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), to induce hypertension, and received losartan or the different hybrids for 3 days. Arterial pressure was measured before and after the treatment with L-NAME and after the

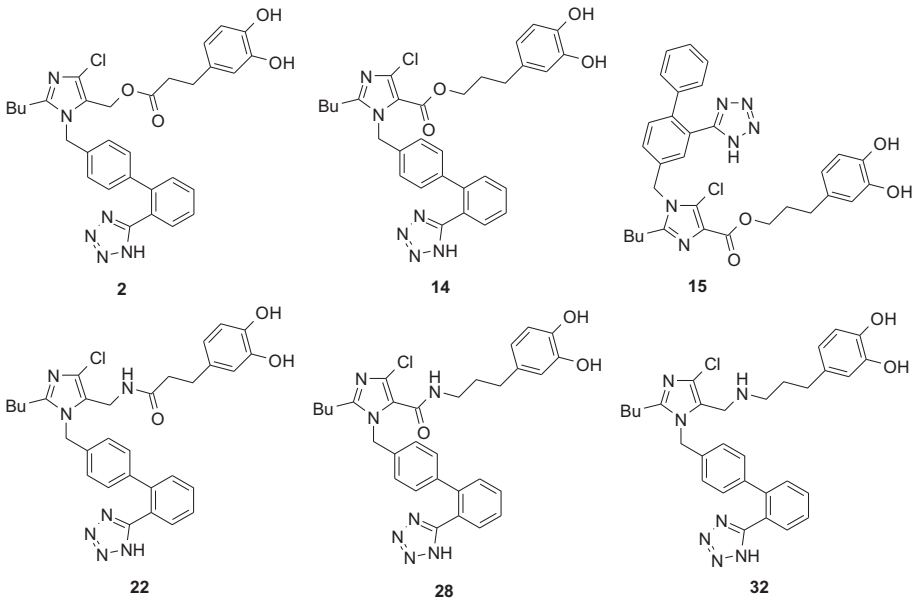


Fig. 4. Structures of hybrids prepared.

treatment with the antihypertensive drug. Then the pressure drop was calculated. Results are shown in Table 3. The more potent antihypertensive was **2** which produced a pressure drop of 2-fold than that of losartan. Ester **15** and amide **22** were antihypertensives as potent as losartan. Ester **14** was slightly less potent than losartan. Unexpectedly amide **28** didn't show any antihypertensive effect. Hybrid amine **32** was not tested.

2.4.3.1.. Structure–activity relationship (SAR) studies. The hierarchical antihypertensive potency order found for the hybrids tested and losartan was: **2** > **15** ≈ **22** ≈ **1** > **14** > **28** (inactive). When losartan is the alcohol moiety of the ester linker (**2** *vs* **14**) the hybrid behaves as a better antihypertensive. The same situation also occurs when losartan is the amine moiety of the amide linker (**22** *vs* **28**). Independently of the position of losartan around the linker, an ester is better accepted than an amide (**2** *vs* **14** and **22** *vs* **28**). The position of the biphenylmethyl side chain also affects the potency (**14** *vs* **15**). If the biphenylmethyl group is linked to the nitrogen *N*-1 atom (**15**) of the imidazole ring the activity is enhanced over the position *N*-3 (**14**).

3. Conclusion

In summary, novel losartan–hydrocaffeic acid hybrids bearing ester, amide and amine linkers between the antihypertensive and antioxidant moieties have been prepared. These new hybrids showed an antioxidant ability ranging from 3 to 9 times than that of **1** or its active metabolite **5**, and were as potent as **1** in inhibiting cellular contraction with values ranging from 88 to 95% inhibition. Most of them showed to be antihypertensives as good as losartan. Biological activities tested showed that **2** was the best hybrid, since it combines both the higher antihypertensive and antioxidant activities. It is better antihypertensive than losartan and may give an extra tissue protection through its antioxidant properties. Hybrids **15** and **22** are also promising candidates as antihypertensive drugs since they produce a drop of arterial pressure as good as losartan and they keep enough antioxidant capacity. Thus, **15** and **22** represent potential hits for further studies. No evidence have been found that any of the products behave as prodrugs, metabolically dissociating into its components.

Structure–activity relationship studies showed that to have a good losartan–antioxidant hybrid the antioxidant phenol must bear at least to hydroxy groups, one of them in *para*-position with respect to the spacer chain. The spacer must have at least a methylene group between the phenol and the linker functional group. The linker must be preferably an ester, the better being losartan the alcohol moiety. Also, an amide is well accepted if losartan provides the amine moiety. Also an ester linker which bears the imidazole ring linked to the carbonyl group is well accepted, but in this case the biphenylmethyl moiety must be linked to the nitrogen *N*-1 of the imidazole ring.

Table 3
Antihypertensive effect of the new hybrids prepared and reference compounds.

Compound	P_0(mm Hg)[a]	P_1 (mm Hg)[b]	P_2 (mm Hg)[c]	P_2–P_1 (mm Hg)
1	126 ± 5	171 ± 3[d]	157 ± 6[d,e]	13 ± 1
2	139 ± 6	175 ± 5[d]	145 ± 2[e]	29 ± 4[f]
14	127 ± 5	175 ± 3[d]	167 ± 3[d,e]	9 ± 2[f]
15	136 ± 5	177 ± 5[d]	163 ± 4[d,e]	14 ± 2
22	131 ± 3	175 ± 2[d]	162 ± 5[d,e]	13 ± 1
28	128 ± 4	171 ± 6[d]	170 ± 5[d]	1 ± 1[f]

[a] Basal systolic arterial pressure.
[b] Systolic arterial pressure after 1-week treatment with L-NAME.
[c] Systolic arterial pressure after 3-day treatment with hybrid drug.
[d] $p < 0.05$ *vs* basal.
[e] $p < 0.05$ *vs* L-NAME.
[f] $p < 0.05$ *vs* losartan.

4. Experimental

4.1. Chemistry

Melting points were determined with a GALLENKAMP apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz and 50 MHz, respectively, on a Varian Gemini 200. Chemical shifts are given in ppm relative to solvent. Signals are quoted as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sext) and multiplet (m). Coupling constants (J) are given in Hertz (Hz). MS were recorded on Hewlett–Packard 5988A or Hewlett–Packard 1100MSD mass spectrometers. Elemental analyses were performed on a Heraeus

CHN Rapid analyzer. FTIR spectra were recorded on a Perkin–Elmer FTIR 1725X spectrophotometer. Reagents and solvents were obtained from commercial sources and used without further purification. TLC was carried out on Alugram Sil G/UV254 silica gel plates. Preparative gravity column chromatography was performed on Merck silica gel. Reported yields are not optimized. Purity of the hybrids and new compounds was determined by elemental analysis (accuracy of within ±0.4%) and HPLC, which indicated a purity >98% for each product.

4.1.1. General hydrogenation method

In a round-bottomed flask the compound to be hydrogenated was dissolved in MeOH or $CHCl_3$/MeOH and 30% Pd–C was added. The flask was capped with a septum and flushed with argon. A balloon containing hydrogen was connected to the flask through a needle and a syringe. The reaction mixture was stirred at room temperature until TLC showed complete conversion of starting material. The mixture was filtered through a short pad of Celite, washed with MeOH and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel.

4.1.2. 3-(3,4-Bis-benzyloxy-phenyl)-propan-1-ol (**6**)

A solution of **4** (0.50 g; 2.74 mmol) and benzyl bromide (2.11 g; 12.34 mmol) in anh. DMF (5 mL) was treated with anh. K_2CO_3 (2.27 g; 16.46 mmol) for 20 h at 60 °C under an argon atmosphere. The reaction mixture was cooled to rt, filtered and evaporated under reduced pressure. The residue was dissolved in AcOEt (10 mL), washed with 1 M HCl (3 × 5 mL), satd. NaCl (5 × 5 mL) and dried ($MgSO_4$). The desiccant was filtered off and the solvent was evaporated under reduced pressure. The residue was dissolved in anh. Et_2O/THF 1:1 (18 mL), added to a slurry of $LiAlH_4$ (0.10 g; 2.71 mmol) in anh. Et_2O at 0 °C and the mixture was stirred at room temperature for 2.5 h. 20% HCl was added to give pH = 1 and the mixture was extracted with AcOEt/Et_2O 1:1 (4 × 10 mL). The organic phase was washed with satd. NaCl and dried (Na_2SO_4). The desiccant was filtered off and the solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel using hexane/AcOEt 1:1. Compound **6** (0.68 g; 56%) was obtained as a colourless oil: Rf = 0.31 (hexane/AcOEt 1/1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.38–7.21 (m, 10H), 6.78 (d, J = 8.2 Hz, 1H), 6.72 (s, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.06 (s, 2H), 5.04 (s, 2H), 3.48 (t, J = 6.2 Hz, 2H), 2.51 (t, J = 7.6 Hz, 2H), 1.70 (quin, 2H) ppm; IR ($CHCl_3$): ν = 3390, 3031, 2937, 1588, 1511, 1453, 1424, 1380, 1262, 1217, 1135, 1023, 909, 849, 808, 755, 696, 666 cm^{-1}; MS (EI, 70 eV) m/z (%) 348 (55) $[M + H]^+$; Anal. calcd for $C_{23}H_{24}O_3$: C 79.28, H 6.94, found: C 79.00, H 6.99.

4.1.3. 2-Butyl-5-chloro-3H-imidazole-4-carboxylic acid ethyl ester (**8**)

To a solution of **7** (3.00 g, 16.07 mmol) in EtOH (120 mL) was added NaCN (4.17 g, 85.2 mmol), AcOH (1.54 g, 25.71 mmol) and MnO_2 (29.34 g, 337.47 mmol). The mixture was stirred for 20 h at 60 °C. After cooling, the mixture was filtered and the solvent was evaporated under reduced pressure. The residue was dissolved in Et_2O (60 mL) and washed with H_2O (30 mL). The organic phase was dried ($MgSO_4$), the desiccant was filtered off and the solvent was evaporated under reduced pressure to give **8** as a dark brown oil (3.20 g, 86%): Rf = 0.21 (hexane/AcOEt/MeOH 4:1:0.05); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 10.71 (bs, 1H), 4.34 (q, J = 7.1 Hz, 2H), 2.70 (t, J = 7.6 Hz, 2H), 1.70 (quin, J = 7.5 Hz, 2H), 1.43–1.22 (m, 5H), 0.89 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 160.3, 152.5, 134.9, 118.3, 61.8, 31.4, 28.8, 23.1, 14.6, 13.9 ppm; IR ($CHCl_3$): ν = 3617, 3270, 3019, 2965, 2400, 1682, 1508, 1423, 1215, 1073, 757, 668 cm^{-1}; MS (EI, 70 eV) m/z (%): 230 (8) $[M + H]^+$, 188 (91) $[M - 42]^+$, 142 (100) $[M - 88]^+$; Anal. Calcd for $C_{10}H_{15}ClN_2O_2$: C 52.06, H 6.55, N 12.14, found: C 51.99, H 6.59, N 12.23.

4.1.4. 2-Butyl-5-chloro-3H-imidazole-4-carboxylic acid (**9**)

To a solution of **8** (0.57 g, 2.49 mmol) in EtOH (20 mL) was added 10 N NaOH (1 mL) and the mixture was heated under reflux for 2.5 h. The solvent was evaporated and the residue was dissolved in H_2O (10 mL) and extracted with Et_2O (10 mL). The aqueous phase was treated with 1 M HCl to give pH = 3–4, extracted with AcOEt (3 × 10 mL) and dried ($MgSO_4$). The desiccant was filtered off and evaporated under reduced pressure to give **9** (0.39 g, 78%) as a dark brown oil: Rf = 0.37 (AcOEt/MeOH 2:1); mp: 192.2–193.0 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 2.69 (t, J = 7.6 Hz, 2H), 1.71 (quin, J = 7.6 Hz, 2H), 1.38 (sext, J = 7.4 Hz, 2H), 0.97 ppm (t, J = 7.4 Hz, 3H). ^{13}C NMR (300 MHz, CD_3OD): δ = 161.5, 152.0, 134.7, 118.6, 31.5, 28.7, 23.1, 14.0 ppm. IR (KBr): ν = 3202, 2959, 2874, 2439, 1942, 1670, 1569, 1518, 1326, 1279, 1238, 1087, 824, 776, 760, 686 cm^{-1}. MS (EI, 70 eV) m/z (%): 202 (8) $[M + H]^+$, 160 (76) $[M - 42]^+$, 142 (100) $[M - 60]^+$. Anal. Calcd for $C_8H_{11}ClN_2O_2$: C 47.41, H 5.47, N 13.82, found: C 47.22, H 5.48, N 13.95.

4.1.5. 2-Butyl-5-chloro-3H-imidazole-4-carboxylic acid 3-(3,4-bis-benzyloxy-phenyl)-propyl ester (**10**)

A solution of **6** (2.00 g, 5.74 mmol) and Ph_3P (1.50 g, 5.74 mmol) in anh. Et_2O (75 mL) was added dropwise to a solution of **9** (1.16 g, 5.74 mmol) and DEAD (0.99 g, 5.74 mmol) in anh. Et_2O/THF 3:1 (100 mL). The reaction mixture was stirred for 12 h at rt. The mixture was filtered and the solvents were removed under reduced pressure. The residue was chromatographed on silica gel using hexane/AcOEt 1:1 to give **10** (2.08 g, 68%) as a yellowish oil: Rf = 0.52 (hexane/AcOEt 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.46 (bs, 1H), 7.43–7.24 (m, 10H), 6.86–6.67 (m, 3H), 5.11 (s, 2H), 5.10 (s, 2H), 4.23 (t, J = 6.2 Hz, 2H), 2.67 (t, J = 7.7 Hz, 4H), 1.97 (quin, J = 6.6 Hz, 2H), 1.69 (quin, J = 7.5 Hz, 2H), 1.34 (sext, J = 7.5 Hz, 2H), 0.90 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 159.9, 151.1, 148.7, 147.2, 137.2, 137.1, 134.3, 128.3, 127.7, 127.2, 121.2, 116.7, 115.6, 115.2, 71.4, 71.3, 64.2, 31.4, 30.1, 29.9, 28.3, 22.2, 13.6 ppm; IR (KBr): ν = 3261, 3018, 2961, 2874, 1680, 1558, 1510, 1454, 1424, 1405, 1327, 1215, 1136, 1073, 1024, 755, 696, 696 cm^{-1}. MS (EI, 70 eV) m/z (%): 555 (100) $[M + Na]^+$; Anal. Calcd for $C_{31}H_{33}ClN_2O_4$: C 69.84, H 6.24, N 15.25, found: C 70.00, H 6.36, N 15.42.

4.1.6. 2-Butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid 3-(3,4-bis-benzyloxy-phenyl)-propyl ester (**12**) and 2-Butyl-5-chloro-1-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-imidazole-4-carboxylic acid 3-(3,4-bis-benzyloxy-phenyl)-propyl ester (**13**)

To a mixture of 10 (1.88 g, 3.53 mmol), tetrabutylphosphonium bromide (0.120 g, 0.353 mmol), 10 N NaOH (0.8 mL), H_2O (2.8 mL) and CH_2Cl_2 (11 mL) was added a solution of **11** (1.96 g, 3.53 mmol) in CH_2Cl_2 (17 mL). The reaction mixture was stirred for 24 h at rt. H_2O (15 mL) was added, the organic phase was separated, washed with H_2O (2 × 15 mL) and dried ($MgSO_4$). The mixture was filtered and the solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel using hexane/AcOEt 2:1. *Compound* **12**. Colourless oil (0.88 g, 25%): Rf = 0.4 (hexane/AcOEt 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.91–7.88 (m, 1H), 7.48–7.21 (m, 22H), 7.08 (d, J = 7.8 Hz, 2H), 6.93–6.65 (m, 11H), 5.44 (s, 2H), 5.11 (s, 2H), 5.10 (s, 2H), 4.12 (t, J = 6.1 Hz, 2H), 2.63 (t, J = 7.4 Hz, 2H), 2.50 (t, J = 7.7 Hz, 2H), 1.90 (quin, J = 6.8 Hz, 2H), 1.64 (quin, J = 7.9 Hz, 2H), 1.27 (sext, J = 7.3 Hz, 2H), 0.85 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 163.9, 159.4, 152.3, 148.8, 147.1, 141.3, 141.1, 140.6, 137.4, 137.2, 136.6, 134.6, 134.5, 130.7, 130.2, 130.1, 129.9, 129.7, 128.3, 128.1, 127.6, 127.5, 127.3, 127.2, 126.1, 125.3, 121.2, 117.1, 115.7, 115.3, 82.8, 71.5, 71.3, 63.8, 48.2, 31.5, 30.2, 29.4, 26.8, 22.3, 13.7 ppm; IR ($CHCl_3$): ν = 3423, 3059, 3030, 2955, 2929, 2869, 1701, 1603, 1511, 1491, 1451, 1424, 1392, 1374, 1264, 1224, 1138, 1103, 1025, 1005, 903, 880, 747, 696 cm^{-1}; MS (APCI) m/z (%): 1008 (100)

$[M + H]^+$; Anal. Calcd for $C_{64}H_{57}ClN_6O_4$: C 76.13, H 5.69, N 8.32, found: C 76.02, H 5.72, N 8.45. *Compound* **13**. Yellowish oil (0.85 g, 24%): Rf = 0.16 (hexane/AcOEt 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.96–7.93 (m, 1H), 7.52–7.21 (m, 22H), 7.11 (d, J = 8.2 Hz, 2H), 6.92–6.69 (m, 11H), 5.12 (s, 2H), 5.11 (s, 2H), 5.03 (s, 2H), 4.33 (t, J = 6.6 Hz, 2H), 2.66 (t, J = 7.6 Hz, 2H), 2.55 (t, J = 7.9 Hz, 2H), 2.04 (quin, J = 7.2 Hz, 2H), 1.61 (quin, J = 7.9 Hz, 2H), 1.27 (sext, J = 7.3 Hz, 2H), 0.83 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 163.8, 161.9, 149.0, 148.8, 147.2, 141.2, 141.15, 141.1, 137.4, 137.3, 134.8, 133.1, 130.6, 130.1, 129.9, 129.8, 128.3, 128.2, 127.7, 127.68, 127.64, 127.5, 127.3, 127.2, 126.8, 126.1, 125.4, 123.4, 121.2, 115.5, 115.3, 82.8, 71.5, 71.3. 63.9. 46.9, 31.6, 30.3, 29.7, 27.6, 22.3, 13.6 ppm; IR ($CHCl_3$): ν = 3422, 3059, 3030, 2954, 2868, 1715, 1603, 1534, 1511, 1449, 1427, 1324, 1262, 1190, 1155, 1136, 1081, 1037, 1005, 903, 880, 847, 817, 783, 747, 697, 677 cm^{-1}. MS (APCI) m/z (%): 1009 (100) $[M + H]^+$; Anal. Calcd for $C_{64}H_{57}ClN_6O_4$: C 76.13, H 5.69, N 8.32, found: C 76.23, H 5.87, N 8.45.

4.1.7. 2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid 3-(3,4-dihydroxy-phenyl)-propyl ester (**14**)

Following the general hydrogenation method, **12** (0.55 g, 0.54 mmol) in MeOH/$CHCl_3$ 7:3 (14 mL) was treated with 30% Pd–C (0.35 g) for 100 min. The residue was triturated with Et_2O and chromatographed using AcOEt/MeOH 2:1 to give **14** (0.22 g, 70%) as a white solid: Rf = 0.55 (AcOEt/MeOH 2:1); mp: 150–151 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.57–7.40 (m, 4H), 7.1 (d, J = 8.2 Hz, 2H), 6.9 (d, J = 8.2 Hz, 2H), 6.68–6.64 (m, 2H), 6.51–6.48 (m, 1H), 5.59 (s, 2H), 4.22 (t, J = 6.2 Hz, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.59 (t, J = 7.4 Hz, 2H), 1.93 (quin, J = 7.1 Hz, 2H), 1.61 (quin, J = 7.1 Hz, 2H), 1.35 (sext, J = 7.3 Hz, 2H), 0.91 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 162.0, 160.6, 154.0, 146.2, 144.4, 142.7, 141.8, 136.4, 134.1, 131.8, 131.3, 130.7, 130.3, 128.2, 126.7, 120.7, 116.5, 116.3, 65.3, 49.4, 32.4, 31.5, 30.5, 27.5, 23.2, 14.0 ppm; IR (KBr): ν = 3179, 2957, 2870, 1701, 1604, 1527, 1486, 1459, 1425, 1397, 1361, 1267, 1208, 1148, 1119, 813, 760, 702, 669 cm^{-1}; MS (APCI) m/z (%): 587 (100) $[M + H]^+$; Anal. Calcd for $C_{31}H_{31}ClN_6O_4$: C 63.42, H 5.32, N 14.31, found: C 63.25, H 5.45, N 14.54.

4.1.8. 2-Butyl-5-chloro-1-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-imidazole-4-carboxylic acid 3-(3,4-dihydroxy-phenyl)-propyl ester (**15**)

Following the general hydrogenation method, **13** (0.71 g, 0.70 mmol) in MeOH/$CHCl_3$ 7:3 (18 mL) was treated with 30% Pd–C (0.45 g) for 75 min. The residue was triturated with Et_2O and chromatographed using AcOEt/MeOH 2:1 to give **15** (0.29 g, 69%) as a white solid: Rf = 0.47 (AcOEt/MeOH 2:1); mp: 177.5–177.7 °C; ^{1}H NMR (200 MHz, CD_3OD): δ = 7.61–7.49 (m, 4H), 7.16 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 7.8 Hz, 2H), 6.69 (d, J = 8.0 Hz, 2H), 6.55 (d, J = 8.0 Hz, 1H), 5.3 (s, 2H), 4.30 (t, J = 6.3 Hz, 2H), 2.72–2.60 (m, 4H), 2.03 (quin, J = 6.7 Hz, 2H), 1.59 (quin, J = 7.7 Hz, 2H), 1.35 (sext, J = 7.5 Hz, 2H), 0.90 ppm (t, J = 7.5 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 163.3, 160.9, 151.3, 146.1, 144.3, 142.5, 141.9, 135.2, 134.2, 131.7, 131.3. 130.9. 130.7, 128.5, 127.8, 127.0, 120.7, 125.0, 120.7, 116.5, 116.3, 65.1, 48.0, 32.4, 31.7, 30.6, 28.1, 23.2, 14.0 ppm; IR (KBr): ν = 3137, 3057, 2957, 2870, 2712, 1699, 1651, 1603, 1531, 1456, 1406, 1360, 1284, 1257, 1210, 1155, 1117, 1088, 1044, 1013, 1006, 865, 842, 816, 783, 758 cm^{-1}; MS (APCI) m/z (%): 587 (100) $[M + H]^+$; Anal. Calcd for $C_{31}H_{31}ClN_6O_4$: C 63.42, H 5.32, N 14.31, found: C 63.21, H 5.32, N 14.02.

4.1.9. 2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carbaldehyde oxime (**18**)

To a solution of **17** (11.83 g, 17.84 mmol) in EtOH (280 mL), $NH_2OH.HCl$ (2.72 g, 39.25 mmol) was added. The reaction mixture was heated for 3 h under reflux. The solvent was evaporated under reduced pressure and the residue was triturated with AcOEt (100 mL) to give **18** (7.69 g, 99%) as a white solid: Rf = 0.06 (hexane/AcOEt 2:1); mp: 215–217 °C; ^{1}H NMR (200 MHz, CD_3OD): δ = 8.11 (s, 1H), 7.75–7.43 (m, 4H), 7.18 (s, 4H), 5.82 (s, 2H), 2.95 (t, J = 7.6 Hz, 2H), 1.61 (quin, J = 7.6 Hz, 2H), 1.38 (sext, J = 7.6 Hz, 2H), 0.92 ppm (t, J = 7.1 Hz, 3H); ^{13}C NMR (200 MHz, CD_3OD): δ = 156.5, 151.6, 142.6, 140.7, 136.9, 135.4, 132.5, 131.7, 131.5, 130.7, 129.1, 127.7, 124.2, 123.9, 122.6, 50.8, 29.8, 26.2, 13.1, 13.8 ppm; IR (KBr): ν = 2999, 1800, 1568, 1476, 1418, 1189, 1152, 1058, 994, 874, 778, 763, 703, 610 cm^{-1}; MS (APCI) m/z (%): 436 (100) $[M + H]^+$; Anal. Calcd for $C_{22}H_{22}ClN_7O$: C 60.61, H 5.08, N 22.49, found: C 60.78, H 5.15, N 22.58.

4.1.10. 2-Butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carbaldehyde O-trityl-oxime (**19**)

A mixture of **18** (4.00 g, 9.17 mmol), TBAI (1.016 g, 2.75 mmol), anh. K_2CO_3 (2.78 g, 20.17 mmol) and Ph_3CCl (7.67 g, 20.17 mmol) in anh. MeCN (220 mL) was heated for 3 h under reflux. The reaction mixture was filtered and the solvent was evaporated. The residue was dissolved in $CHCl_3$ (100 mL), washed with satd. NaCl (2 × 100 mL), H_2O (2 × 100 mL) and dried (Na_2SO_4). The mixture was filtered and the solvent was evaporated to give a yellowish oil, which was chromatographed on silica gel using hexane/AcOEt 3:1. The resulting oil was triturated with Et_2O to give **19** (2.98 g, 48%) as a white solid: Rf = 0.43 (hexane/AcOEt 3:1); mp: 208–210 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.38 (s, 1H), 7.97 (dd, J = 1.4 Hz, J = 7.3 Hz, 1H), 7.65–7.53 (m, 2H), 7.46–7.21 (m, 25H), 7.05–7.00 (m, 8H), 6.49 (d, J = 8.2 Hz, 2H), 4.99 (s, 2H), 2.32 (t, J = 7.6 Hz, 2H), 1.61 (quin, J = 7.3 Hz, 2H), 1.23 (sext, J = 7.6 Hz, 2H), 0.86 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$): δ = 166.0, 153.3, 146.0, 143.5, 143.2, 141.9, 140.3, 136.5, 134.9, 132.8, 132.5, 132.1, 131.9, 131.4, 131.2, 130.9, 130.2, 129.8, 129.6, 129.4, 129.2, 129.0, 128.4, 128.2, 127.2, 121.3, 93.3, 84.8, 50.2, 31.3, 28.4, 24.2, 15.7 ppm; IR (KBr): ν = 3059, 3010, 2960, 2872, 1613, 1520, 1491, 1448, 1410, 1264, 1216, 1187, 1156, 1033, 1003, 979, 962, 925, 879, 755, 699, 667, 633 cm^{-1}; MS (APCI) m/z (%): 921 (3) $[M + H]^+$, 865 (14) $[M - 55 + H]^+$; Anal. Calcd for $C_{60}H_{50}ClN_7O$: C 78.28, H 5.47, N 10.65, found: C 78.54, H 5.57, N 10.85.

4.1.11. {2-Butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-yl}-methylamine (**20**)

A solution of **19** (1.93 g, 2.1 mmol) in anh. THF (40 mL) was added to a slurry of $LiAlH_4$ (1.19 g, 31.5 mmol) in anh. THF (40 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 3.5 h at rt. Satd. NH_4Cl was added and the mixture was extracted with AcOEt (2 × 40 mL) and dried (Na_2SO_4). The desiccant was filtered off and the solvent was evaporated under reduced pressure. A yellowish oil was obtained and this was chromatographed on silica gel using first hexane/AcOEt 1:1 and then AcOEt/MeOH 5:1 to give **20** (0.60 g, 43%) as a white foam: Rf = 0.43 (AcOEt/MeOH 5:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.93–7.89 (m, 1H), 7.51–7.38 (m, 2H), 7.36–7.20 (m, 10H), 7.09 (d, J = 7.9 Hz, 2H), 6.92–6.87 (m, 6H), 6.72 (d, J = 7.9 Hz, 2H), 5.12 (s, 2H), 3.49 (s, 2H), 2.47 (t, J = 7.7 Hz, 2H), 1.63 (quin, J = 7.9 Hz, 2H), 1.43 (bs, 2H), 1.26 (sext, J = 7.6 Hz, 2H), 0.83 ppm (t, J = 7.6 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$): δ = 163.8, 147.5, 141.2, 140.8, 134.8, 130.6, 130.2, 130.1, 129.9, 129.8, 128.2, 127.6, 127.6, 126.2, 126.1, 125.7, 125.0, 82.8, 46.9, 34.6, 29.8, 22.4, 13.8 ppm; IR ($CHCl_3$): ν = 3060, 3010, 2958, 2932, 2871, 1600, 1577, 1493, 1447, 1425, 1410, 1356, 1250, 1216, 1157, 1029, 1005, 932, 880, 754, 698 cm^{-1}. MS (ES+) m/z (%): 664 (22) $[M + H]^+$; Anal. calcd for: $C_{41}H_{38}ClN_7$: C 74.13, H 5.76, N 14.76, found: C 74.02, H 5.24, N 14.59.

4.1.12. 3-(3,4-Bis-benzyloxy-phenyl)-N-{2-butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl}-propionamide (**21**)

A solution of CDI (0.118 g, 0.72 mmol) in anh. THF (3 mL) was added to a slurry of **16** (0.12 g, 0.33 mmol) in anh. THF (3 mL) under

an argon atmosphere. The reaction mixture was heated for 20 h at 60 °C. A solution of 20 (0.22 g, 0.33 mmol) in anh. THF (3 mL) was added and the reaction mixture was stirred for 4 h at 60 °C. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ (5 mL). The organic phase was washed with satd. NaCl (5 mL) and the aqueous phase was extracted with $CHCl_3$ (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual oil was chromatographed on silica gel using hexane/AcOEt 1:2 to give **21** (0.19 g, 58%) as a yellowish oil: Rf = 0.47 (hexane/AcOEt 1:2); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.87–7.83 (m, 1H), 7.39–7.14 (m, 22H), 7.03 (d, J = 8.2 Hz, 2H), 6.91–6.87 (m, 6H), 6.74–6.61 (m, 4H), 6.54–6.49 (m, 1H), 5.23 (t, J = 5.7 Hz, 1H), 5.06 (s, 2H), 5.01 (s, 2H), 5.00 (s, 2H), 4.1 (d, J = 5.7 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 2.40 (t, J = 7.7 Hz, 2H), 2.02 (t, J = 7.6 Hz, 2H), 1.0 (quin, J = 7.6 Hz, 2H), 1.20 (sext, J = 7.3 Hz, 2H), 0.79 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$): δ = 171.6, 163.8, 148.7, 148.0, 147.3, 141.2, 141.1, 140.7, 137.3, 137.2, 134.8, 133.8, 130.6, 130.2, 130.1, 129.9, 129.7, 128.3, 128.2, 127.6, 127.2, 127.2, 126.1, 124.8, 122.4, 120.9, 115.3, 115.2, 82.8, 71.3, 71.2, 46.8, 37.8, 31.5, 30.7, 29.7, 26.7, 22.3, 13.7 ppm; IR ($CHCl_3$): ν = 3293, 3062, 3010, 2958, 2931, 2871, 1662, 1511, 1450, 1424, 1257, 1216, 1016, 1005, 755, 697 cm^{-1}; MS (APCI) m/z (%): 1008 (100) $[M + H]^+$; Anal. calcd for: $C_{64}H_{58}ClN_7O_3$: C 76.21, H 5.79, N 9.72, found: C 76.05, H 5.70, N 9.65.

4.1.13. *N-{2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl}-3-(3,4-dihydroxy-phenyl)-propionamide (**22**)*

Following the general hydrogenation method, **21** (0.44 g, 0.44 mmol) in MeOH/$CHCl_3$ 7:3 (9 mL) and 30% Pd–C (0.33 g) were stirred for 45 min at rt. The residue was chromatographed using AcOEt/MeOH 5:1 to give **22** (0.18 g, 68%) as a white solid; Rf: 0.31 (AcOEt/MeOH 5:1); mp: 178.4–178.6 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.58 (dd, J = 1.5 Hz, J = 7.1 Hz 1H), 7.54–7.44 (m, 2H), (dd, J = 1.4 Hz, J = 7.7 Hz 1H), 7.1 (d, J = 8.2 Hz, 2H), 6.8 (d, J = 8.0 Hz, 2H), 6.64–6.60 (m, 2H), 6.44 (dd, J = 2.1 Hz, J = 7.8 Hz 1H), 5.02 (s, 2H), 4.28 (s, 2H), 2.62 (t, J = 7.5 Hz, 2H), 2.55 (t, J = 7.6 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 1.56 (quin, J = 7.5 Hz, 2H), 1.32 (sext, J = 7.5 Hz, 2H), 0.89 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 174.9, 160.7, 149.9, 146.1, 144.5, 142.6, 141.4, 136.5, 133.4, 131.7, 131.4, 130.8, 130.7, 128.7, 128.4, 127.8, 126.5, 124.2, 120.5, 116.5, 116.2, 47.9, 38.4, 32.3, 31.8, 30.8, 27.3, 23.2, 14.0 ppm; IR (KBr): ν = 3252, 3059, 2957, 2870, 2715, 1650, 1604, 1527, 1459, 1424, 1360, 1258, 1209, 1152, 1116, 1075, 1005, 819, 778, 759 cm^{-1}; MS (APCI) m/z (%): 586 $[M + H]^+$; Anal. calcd for: $C_{31}H_{32}ClN_7O_3$: C 63.52, H 5.50, N 16.72, found: C 63.25, H 5.48, N 16.62.

4.1.14. *2-[3-(3,4-Bis-benzyloxy-phenyl)-propyl]-isoindole-1,3-dione (**23**)*

To a solution of **6** (2.58 g, 7.41 mmol), Ph_3P (1.94 g, 7.41 mmol) and phthalimide (1.09 g, 7.41 mmol) in anh. THF (60 mL) was added DEAD (0.174 g, 7.41 mmol) under an argon atmosphere. The reaction mixture was stirred for 20 h at rt. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel using hexane/AcOEt 1:1 to give an oil, which was triturated in Et_2O to yield **23** (0.22 g; 94%) as a white solid: Rf = 0.72 (AcOEt/MeOH 1:1); mp: 91–93 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81–7.70 (m, 2H), 7.69–7.66 (m, 2H), 7.45–7.26 (m, 10H), 6.81–6.78 (m, 2H), 6.69–6.66 (m, 1H), 5.12 (s, 2H), 5.05 (s, 2H), 3.68 (t, J = 7.0 Hz, 2H), 2.57 (t, J = 7.6 Hz, 2H), 1.95 ppm (quin, J = 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.3, 148.8, 147.1, 137.4, 137.3, 134.5, 133.8, 132.0, 128.3, 127.6, 127.3, 127.2, 123.0, 121.0, 115.3, 71.4, 71.1, 37.6, 32.6, 29.7 ppm; IR ($CHCl_3$): ν = 3461, 3061, 3030, 2934, 2863, 1779, 1769, 1713, 1607, 1587, 1517, 1465, 1454, 1436, 1427, 1398, 1380, 1365, 1331, 1266, 1245, 1224, 1188, 1136, 1106, 1083, 1027, 1006, 969, 944, 914, 886, 855, 791, 766, 733, 708, 698, 605 cm^{-1}; MS (APCI) m/z (%): 500 (100) $[M + Na]^+$; Anal. calcd. for: $C_{31}H_{27}NO_4$: C 77.96, H 5.69, N 2.93, found: C 78.00; H 5.71, N 3.00.

4.1.15. *3-(3,4-Bis-benzyloxy-phenyl)-propylamine (**24**)*

To a solution of **23** (3.12 g, 6.54 mmol) in EtOH (30 mL) was added hydrazine monohydrate (0.32 g, 6.54 mmol) and the mixture was heated for 10 h under reflux. The solvent was removed under reduced pressure and the residue was triturated with $CHCl_3$. The solid was filtered off and the filtrate was evaporated under reduced pressure. The residual oil was chromatograped on silica gel using MeOH to give **24** (1.47 g, 65%) as a yellowish oil: Rf = 0.17 (MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48–7.25 (m, 10H), 6.89–6.80 (m, 2H), 6.70 (dd, J = 1.8 Hz, J = 8.0 Hz, 1H), 5.15 (s, 2H), 5.12 (s, 2H), 2.65 (t, J = 7.1 Hz, 2H), 2.55 (t, J = 7.7 Hz, 2H), 1.69 (quin, J = 7.3 Hz, 2H), 1.34 ppm (bs, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 148.7, 147.1, 137.5, 137.3, 135.6, 128.3, 127.6, 127.6, 127.29, 127.2, 121.1, 115.6, 115.3, 71.5, 71.3, 41.6, 35.3, 32.6 ppm; IR ($CHCl_3$): ν = 3031, 2929, 2858, 1587, 1511, 1453, 1424, 1380, 1263, 1223, 1137, 1079, 1023, 852, 806, 735, 696 cm^{-1}; MS (APCI) m/z (%): 348 (100) $[M + H]^+$; Anal. calcd for: $C_{23}H_{25}NO_2$: C 79.50, H 7.25, N 4.03, found: C 79.25, H 7.52, N 4.08.

4.1.16. *2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid (**5**) and 2-butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carbaldehyde (**26**)*

To a solution of **17** (2.00 g, 3.01 mmol) in EtOH (23 mL) was added NaCN (0.78 g, 16 mmol), AcOH (0.29 g, 4.81 mmol) and MnO_2 (5.50 g, 63.21 mmol) and the mixture was stirred for 21 h under reflux. The reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was dissolved in Et_2O (12 mL), washed with H_2O (12 mL) and dried ($MgSO_4$). The desiccant was filtered off and the solvent was removed under reduced pressure to give a dark brown oil. The oil was dissolved in EtOH (45 mL), 10 N NaOH (1 mL) was added and the mixture was heated for 3 h under reflux. The solvent was removed under reduced pressure, the residue was dissolved in H_2O (10 mL) and 1 M HCl was added to give pH = 3–4. The mixture was extracted with AcOEt (3 × 10 mL) and the combined organic extracts were dried ($MgSO_4$). The mixture was filtered and the solvent was evaporated under reduced pressure to give a dark brown oil, which was chromatographed on silica gel using AcOEt/MeOH 2:1. *Compound* **5**: yellowish solid (1.02 g, 78%); Rf = 0.28 (AcOEt/MeOH 2:1); mp: 177–179 °C (Lit [35]: 176–178 °C); ^{1}H NMR (200 MHz, MeOD): δ = 7.60–7.40 (m, 4H), 7.07 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 5.72 (s, 2H), 2.62 (t, J = 7.5 Hz, 2H), 1.56 (quin, 2H), 1.32 (sext, 2H), 0.88 ppm (t, J = 7.1 Hz, 3H); IR (KBr): ν = 3365, 2957, 1599, 1458, 1407, 1359, 1261, 1173, 1108, 1054, 1006, 976, 894, 816, 760, 668 cm^{-1}; MS (ES+) m/z (%): 437 (100) $[M + H]^+$; Anal. calcd for: $C_{22}H_{21}ClN_6O_2$: C 60.48, H 4.84, N 19.23, found: C 60.52, H 4.95, N 19.25. *Compound* **26**: white solid (0.25 g, 20%); mp: 155–157 °C (Lit [35]: 154–155 °C).

4.1.17. *2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid [3-(3,4-bis-benzyloxy-phenyl)-propyl]-amide (**27**)*

A solution of CDI (0.41 g, 2.5 mmol) in anh. THF (15 mL) was added to a slurry of **5** (0.5 g, 1.14 mmol) in anh. THF (40 mL) under an argon atmosphere. The mixture was heated for 20 h at 60 °C. A solution of **24** (0.87 g, 2.50 mmol) in anh. THF (15 mL) was added and the mixture was heated for 4 h at 60 °C. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ (40 mL) and washed with satd. NaCl (40 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 40 mL) and the combined

organic extracts were dried ($MgSO_4$). The desiccant was filtered off, the solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel using AcOEt/MeOH 2:1 to give **27** (0.23 g, 26%) as a yellow solid that was crystallized from hexane/AcOEt 1:1: Rf = 0.73 (AcOEt/MeOH 1:1); mp: 134–136 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.58–7.26 (m, 14H), 7.08 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 8.2 Hz, 2H), 6.93–6.88 (m, 2H), 6.72–6.69 (m, 1H), 5.47 (s, 2H), 5.10 (s, 2H), 5.08 (s, 2H), 3.27 (t, J = 6.8 Hz, 2H), 2.68 (t, J = 7.7 Hz, 2H), 2.55 (t, J = 7.6 Hz, 2H), 1.77 (quin, J = 7.3 Hz, 2H), 1.61 (quin, J = 7.5 Hz, 2H), 1.37 (sext, J = 7.3 Hz, 2H), 0.92 ppm (t, J = 7.3 Hz, 3H); IR ($CHCl_3$): ν = 3123, 3032, 2931, 2860, 2609, 1958, 1648, 1511, 1258, 1135, 1063, 1013, 930, 852, 751, 696, 663 cm^{-1}; MS (ES+) m/z (%): 766 (100) $[M + H]^+$; Anal. calcd for $C_{45}H_{44}ClN_7O_3$: C 70.52, H 5.78, N 12.79, found: C 69.72, H 5.74, N 12.25.

4.1.18. 2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carboxylic acid [3-(3,4-dihydroxy-phenyl)-propyl]-amide (**28**)

Following the general hydrogenation method, **27** (0.20 g, 0.26 mmol) in MeOH (5 mL) and 30% Pd–C (0.94 g) were stirred for 1 h. The residue was chromatographed using AcOEt/MeOH 2:1 to give **28** (0.10 g, 65%) as a yellowish solid: Rf = 0.49 (AcOEt/MeOH 2:1); mp: 180.2–181.6 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.56–7.32 (m, 4H), 7.09 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 8.2 Hz, 2H), 6.68–6.64 (m, 2H), 6.51–6.48 (m, 1H), 5.46 (s, 2H), 3.29 (t, J = 6.8 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H), 2.49 (t, J = 7.7 Hz, 2H), 1.76 (quin, J = 7.3 Hz, 2H), 1.61 (quin, J = 7.5 Hz, 2H), 1.36 (sext, J = 7.5 Hz, 2H), 0.92 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 162.2, 161.3, 152.0, 146.1, 144.3, 142.6, 142.1, 136.2, 134.5, 131.8, 131.2, 130.7, 130.5, 130.2, 129.8, 128.2, 127.2, 123.0, 120.6, 116.5, 116.2, 40.1, 33.5, 32.3, 30.7, 27.3, 23.2, 14.0 ppm; IR (KBr): ν = 3061, 2957, 2870, 1646, 1515, 1455, 1361, 1263, 1197, 1114, 1006, 814, 777, 760 cm^{-1}. MS (ES+) m/z (%): 586 (100) $[M + H]^+$; Anal. calcd for $C_{31}H_{32}ClN_7O_3$: C 63.52, H 5.50, N 16.72, found: C 63.75, H 5.74, N 16.45.

4.1.19. [3-(3,4-Bis-benzyloxy-phenyl)-propyl]-{2-butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl}-amine (**29**) and {2-Butyl-5-chloro-3-[2′-(2-trityl-2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-yl}-methanol (**31**)

To a solution of **17** (0.90 g, 1.35 mmol) and **24** (0.471 g, 1.35 mmol) in CH_2Cl_2 (36 mL) was added anh. $MgSO_4$ (0.16 g) and the mixture was stirred for 17 h at rt under an argon atmosphere. The mixture was filtered and the solvent was removed under reduced pressure. The residue was dissolved in anh. MeOH/CH_2Cl_2 2:1 (36 mL) under an argon atmosphere, $NaBH_4$ (0.10 g, 2.71 mmol) was added slowly and the mixture was stirred for 4 h at rt. The solvent was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (18 mL), washed with satd. NaCl (2 × 10 mL) and dried ($MgSO_4$). The desiccant was filtered off and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel using hexane/AcOEt 1:2. *Compound* **29**: colourless oil (0.46 g, 34%); Rf = 0.25 (hexane/AcOEt 1:2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95–7.92 (m, 1H), 7.47–7.22 (m, 22H), 7.09 (d, J = 8.2 Hz, 2H), 6.94–6.91 (m, 6H), 6.83 (d, J = 8.0 Hz 2H), 6.73 (d, J = 7.8 Hz 2H), 6.62 (dd, J = 1.9 Hz, J = 8.2 Hz 1H), 5.14 (s, 2H), 5.11 (s, 4H), 3.44 (s, 2H), 2.46 (t, J = 7.7 Hz, 6H), 1.63 (sext, J = 7.7 Hz, 4H), 1.28 (sext, J = 7.4 Hz, 2H), 1.06 (bs, 1H), 0.85 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.9, 148.8, 147.6, 147.1, 141.28, 141.2, 140.7, 137.4, 137.3, 135.5, 134.9, 130.6, 130.2, 130.1, 129.9, 129.7, 128.3, 128.2, 127.7, 127.6, 127.3, 127.2, 127.1, 126.1, 125.0, 123.6, 121.1, 115.6, 115.2, 82.8, 71.5, 71.3, 48.3, 46.8, 41.8, 32.9, 31.5, 29.7, 26.7, 22.4, 13.7 ppm; IR ($CHCl_3$): ν = 3029, 2930, 1586, 1510, 1450, 1379, 1248, 1157, 1136, 1026, 930, 904, 880, 747, 696 cm^{-1}; MS (APCI) m/z (%): 995 (100) $[M + H]^+$; Anal. calcd for $C_{64}H_{60}ClN_7O_2$: C 77.28, H 6.08, N 9.85, found: C 77.12, H 6.14, N 9.99. *Compound* **31**: white solid (0.36 g, 40%); mp: 165–167 °C (Lit [9]: 167–169 °C).

4.1.20. 4-[3-({2-Butyl-5-chloro-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl}-amino)-propyl]-benzene-1,2-diol (**32**) and 4-[3-({2-Butyl-3-[2′-(2H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl}-amino)-propyl]-benzene-1,2-diol (**33**)

Following the general hydrogenation method, **29** (0.19 g, 0.21 mmol) in MeOH/$CHCl_3$ 77:23 (13 mL), and 30% Pd–C (0.17 g) were stirred for 160 min. The residue was chromatographed using AcOEt/MeOH/TEA (2:1:0.2). *Compound* **32**: Orange solid (0.05 g, 40%): Rf = 0.36 (AcOEt/MeOH/TEA 2:1:0.2); mp: 97–99 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.59–7.41 (m, 4H), 7.13 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 6.8 Hz, 2H), 6.68–6.62 (m, 2H), 6.48–6.45 (m, 1H), 5.27 (s, 2H), 3.73 (s, 2H), 2.68–2.63 (m, 4H), 2.47 (t, J = 7.5 Hz, 2H), 1.71 (quin, J = 7.3 Hz, 2H), 1.62 (quin, J = 7.3 Hz, 2H), 1.35 (sext, J = 7.0 Hz, 2H), 0.91 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 162.6, 150.3, 146.1, 144.3, 142.4, 135.9, 134.3, 131.7, 131.1, 131.0, 130.0, 128.9, 128.2, 126.4, 123.3, 120.6, 116.4, 116.2, 48.8, 48.2, 41.3, 33.6, 31.4, 30.9, 27.4, 23.2, 14.0 ppm; IR (KBr): ν = 3381, 2925, 2139, 1636, 1457, 1357, 1257, 1207, 1150, 1119, 1013, 897, 812, 785, 759 cm^{-1}; MS (ES) m/z (%): 572 (100) $[M + H]^+$; Anal. calcd for $C_{31}H_{34}ClN_7O_2$: C 65.08, H 5.99, N 17.13, found: C 65.12, H 6.02, N 17.25. *Compound* **33**. Yellow solid (0.02 g, 15%): Rf = 0.12 (AcOEt/MeOH/TEA 2:1:0.2); mp: 100–101 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.63–7.43 (m, 4H), 7.14–7.11 (m, 3H), 6.82 (d, J = 8.0 Hz, 2H), 6.71–6.65 (m, 2H), 6.53–6.50 (m, 1H), 5.27 (s, 2H), 3.99 (s, 2H), 2.94 (t, J = 7.7 Hz, 2H), 2.74 (t, J = 7.6 Hz, 2H), 2.57 (t, J = 7.4 Hz, 2H), 1.90 (quin, J = 7.7 Hz, 2H), 1.64 (quin, J = 7.0 Hz, 2H), 1.37 (sext, J = 7.4 Hz, 2H), 0.92 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (300 MHz, CD_3OD): δ = 162.6, 152.5, 146.4, 144.8, 142.6, 142.4, 135.7, 133.1, 131.7, 131.1, 130.9, 130.1, 129.3, 128.4, 126.4, 120.5, 116.5, 116.4, 47.7, 41.8, 32.9, 31.0, 29.4, 27.5, 23.3, 14.0 ppm; IR (KBr): ν = 3418, 2957, 2869, 2349, 2297, 1603, 1519, 1459, 1407, 1358, 1282, 1151, 1118, 1011, 955, 889, 820, 786, 760 cm^{-1}. MS (API-ES+) m/z (%): 538 (100) $[M + H]^+$; Anal. calcd for: $C_{31}H_{35}N_7O_2$: C 69.25, H 6.56, N 17.13, found: C 69.42, H 6.75, N 17.21.

4.2. Antioxidant activity of phenols and hybrids

The antioxidant ability of the different compounds was evaluated with a commercial kit (*Total antioxidant status assay kit*) purchased from Calbiochem (La Jolla, CA, USA) [29]. The assay relies on the ability of antioxidants to inhibit the oxidation of a chromogen (ABTS) by metmyoglobin and hydrogen peroxide. Oxidation is monitored by reading the absorbance at 600 nm. The antioxidant ability is proportional to the reduction of the absorbance, and it is expressed as mM. Experimental protocol was modified slightly. A 96-well plate was used instead of a cuvette. Lower volume of chromogen (metmyoglobin and ABTS; 250 μL), water (5 μL), standard (Trolox, 5 μL) and sample solutions (10^{-1} M in PBS, 5 μL) were used. Phenols stock solutions were prepared dissolving 5 mg of sample in 1 equivalent volume of 1.622 M K_2CO_3 and then diluted up to assay concentrations. Two wells were used for each sample. Initial absorbance (A_0) was red after mixing solutions. Then, substrate solution (200 μL) was added, solutions were mixed and absorbance (A) was red after exactly 3 min. The mean of 3 (phenols) or 8 (hybrids) assays was used to calculate TEAC as in Eq. (1) ($\Delta = A - A_0$).

$$\mathrm{TEAC(mM)} = [\mathrm{Standard}](\Delta A\mathrm{Blank} - \Delta A\mathrm{Sample})/ \times (\Delta A\mathrm{Blank} - \Delta A\mathrm{Standard}) \quad (1)$$

4.3. Inhibition of cellular contraction

Vascular smooth muscle cells (VSMC) were obtained from thoracic aortas of Wistar rats by methods described previously [42]. Briefly, thoracic aortas from Wistar rats were removed, cleaned, dissected into small strips, and incubated in DMEM/Ham's F-12 medium (Biowhittaker, Walkersville, MD, USA) with collagenase type IV at 37 °C for 45 min. The digested strips were seeded onto dishes and maintained in 10 mL of DMEM/Ham's F-12 medium with 10% foetal calf serum (Biowhittaker), at 37 °C, in a humidified atmosphere of 5% CO_2. Culture media were changed every 3 days. The primary cultures on days 20–22 were passaged by trypsinization (trypsin–EDTA). The cells were used between the third and fifth passages.

The ability of the different compounds to block angiotensin II-induced cell contraction was performed by studying the changes in planar cell surface area (PCSA) under different experimental conditions [42]. In every experiment, cells were washed and placed in Tris–glucose buffer (20 mM Tris, 130 mM NaCl, 5 mM KCl, 10 mM sodium acetate, and 5 mM glucose, pH 7.45) containing 2.5 mM $CaCl_2$. Cells were incubated with 1 μM angiotensin II, in the presence of **1** or the different synthesized compounds, for 30 min. Photographs (TMS-F Photomicroscope, Nikon, Tokyo, Japan, magnification 150×) of the same cells were taken at times 0 and 30 min. PCSA was determined by computer-aided planimetric techniques. Each experiment included between 4 and 8 cells. The mean of 5 experiments was used to calculate the percentage of cell contraction inhibition.

4.4. Antihypertensive activity

Two-month-old male Wistar rats were treated with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) in the drinking water (20 mg/kg/day). One week after starting L-NAME treatment, some rats were treated with losartan (20 mg/kg/day), **2**, **14**, **15**, **22** or **28** (30 mg/kg/day) for three days. These treatments were administered daily also in the drinking water. At least six animals were included in each experimental group. Arterial pressure was monitored in conscious animals with a tail cuff sphygmomanometer (LE 5001 Pressure Metre, Letica Scientific Instruments, Hospitalet, Spain) [43].

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References

[1] C.A.M. Fraga, Expert Opin. Drug Dis. 4 (2009) 605–609.
[2] B. Meunier, Acc. Chem. Res. 41 (2008) 69–77.
[3] V. Pokrovskaya, T. Baasov, Expert Opin. Drug Dis. 5 (2010) 883–902.
[4] J.B. Bremmer, J.I. Ambrus, S. Samosorn, Curr. Med. Chem. 14 (2007) 1459–1477.
[5] F.W. Muregi, A. Ishih, Drug Develop. Res. 71 (2010) 20–32.
[6] L.K. Gediya, V.C.O. Njar, Expert Opin. Drug Dis. 4 (2009) 1099–1111.
[7] A. Martelli, M.C. Breschi, V. Calderone, Curr. Pharm. Design 15 (2009) 614–636.
[8] A. Gere, E. Agai-Csongor, T. Gizur, G. Domany, Med. Cherm. Res. 7 (1997) 192–198.
[9] D.J. Carini, J.V. Duncia, P.E. Aldrich, A.T. Chiu, A.L. Jonson, M.E. Pierce, W.A. Price, J.B. Santella III, G.J. Wells, R.R. Wexler, P.C. Wong, S.-E. Too, P.B.M.W.M. Timmermans, J. Med. Chem. 34 (1991) 2525–2547.
[10] R.R. Wexler, W.J. Greenlee, J.D. Irvin, M.R. Goldberg, K. Prendergast, R.D. Smith, P.B.M.W.M. Timmermans, J. Med. Chem. 39 (1996) 625–656.
[11] A. Daugherty, L. Cassis, Trends Cardiovasc. Med. 14 (2004) 117–120.
[12] M.C. Breschi, V. Calderone, M. Digiacomo, M. Macchia, A. Martelli, E. Martinotti, F. Minutolo, S. Rapposelli, A. Rossello, L. Testai, A. Balsano, J. Med. Chem. 49 (2006) 2628–2639.
[13] R. Stocker, J.J. Keaney, Physiol. Rev. 84 (2004) 1381–1478.
[14] S. Rajagopalan, X.P. Meng, S. Ramasamy, D.G. Harrison, Z.S. Galis, J. Clin. Invest. 98 (1996) 2572–2579.
[15] M. Ushio-Fukai, R.W. Alexander, M. Akers, Q. Yin, Y. Fujio, K. Walsh, K.K. Griendling, J. Biol. Chem. 274 (1999) 22699–22704.
[16] S. Uemura, H. Matsushita, W. Li, A.J. Glassford, T. Asagami, K.H. Lee, D.G. Harrison, P.S. Tsao, Circ. Res. 88 (2001) 1291–1298.
[17] J.W. Zmijewski, D.R. Moellering, C. Le Goffe, A. Landar, A. Ramachandran, V.M. Darley-Usmar, Am. Physiol. Heart Circ. Physiol. 2 (2005) 852–861.
[18] H. Cai, D.G. Harrison, Circ. Res. 87 (2000) 840–844.
[19] J.S. Pober, W. Min, J.R. Bradley, Annu. Rev. Pathol. 4 (2009) 71–95.
[20] E. Schulz, T. Jansen, P. Wenzel, A. Daiber, T. Münzel, Antioxid. Redox Signal 10 (2008) 1115–1126.
[21] P.F. Li, R. Dietz, R. von Harsdorf, Circulation 96 (1997) 3602–3609.
[22] R. Locher, R.P. Brandes, W. Vetter, M. Barton, Hypertension 39 (2002) 645–650.
[23] H. Okada, J. Woodcock-Mitchell, J. Mitchell, T. Sakamoto, K. Marutsuka, B.E. Sobel, S. Fujii, Circulation 97 (1998) 2175–2182.
[24] M. Bartosz, J. Kedziora, G. Bartosz, Free Radic. Biol. Med. 23 (1997) 729–735.
[25] S. Aslam, T. Santha, A. Leone, C. Wilcox, Kidney Int. 70 (2006) 2109–21015.
[26] T.C. Wu, Y.H. Chen, H.B. Leu, Y.L. Chen, F.Y. Lin, S.J. Lin, J.W. Chen, Free Radic. Biol. Med. 43 (2007) 1508–15022.
[27] G. García, M. Rodríguez-Puyol, R. Alajarín, I. Serrano, P. Sánchez-Alonso, M. Griera, J.J. Vaquero, D. Rodríguez-Puyol, J. Álvarez-Builla, M.L. Díez-Marqués, J. Med. Chem. 52 (2009) 7220–7227.
[28] D. Huang, B. Ou, R.L. Prior, J. Agric. Food Chem. 53 (2005) 1841–1856.
[29] N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, Clin. Sci. 84 (1993) 407.
[30] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radic. Biol. Med. 26 (1999) 1231–1237.
[31] B.F. Abdel-Wahab, G.E.A. Awad, F.A. Badria, Eur. J. Med. Chem. 46 (2011) 1505–1511.
[32] C. Siquet, F. Paiva-Martins, J.L.F.C. Lima, S. Reis, F. Borges, Free Radic. Res. 40 (2006) 433–442.
[33] E. Weber, I. Csöregh, B. Stensland, M. Czugler, J. Am. Chem. Soc. 106 (1984) 3297–3306.
[34] O. Mitsunobu, Synthesis (1981) 1–28.
[35] V. Santagada, F. Fiorino, E. Perissutti, B. Severino, S. Terracciano, C.E. Teixeira, G. Caliendo, Tetrahedron Lett. 44 (2003) 1149–1152.
[36] P. Zoumpoulakis, S.G. Grdadolnik, J. Matsoukas, T. Mavromoustakos, J. Pharm. Biomed. Anal. 28 (2002) 125–135.
[37] T. Mavromoustakos, A. Kolocouris, M. Zervou, P. Roumelioti, J. Matsoukas, R. Weisemann, J. Med. Chem. 42 (1999) 1714–1722.
[38] S.-E. Yoo, S.-K. Kim, S.-H. Lee, N.-J. Kim, D.-W. Lee, Bioorg. Med. Chem. 8 (2000) 2311–2316.
[39] M.W. Lo, M.R. Goldberg, J.B. McCrea, H. Lu, C.I. Furtek, T.D. Bjornsson, Clin. Pharmacol. Ther. 58 (1995) 641–649.
[40] T. Toshiaki, N. Akira, K. Shoji, A. Yawham, Y. Masanori, H. Hitoshi, M. Kyoji, A. Youichi, CardioVasc. Drug Rev. 15 (1997) 122–136.
[41] B. Michel, Circulation 103 (2001) 904–912.
[42] G. Torrecillas, M.C. Boyano-Adanez, J. Medina, T. Parra, M. Griera, S. Lopez-Ongil, E. Arilla, M. Rodríguez-Puyol, D. Rodríguez-Puyol, Mol. Pharmacol. 59 (2001) 104–112.
[43] G. Pérez-Rivero, M.P. Ruiz-Torres, J.V. Rivas-Elena, M. Jerkic, M.L. Díez-Marqués, J.M. López-Novoa, M.A. Blasco, D. Rodríguez-Puyol, Circulation 114 (2006) 309–317.