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NO-Sartans: A New Class of Pharmacodynamic Hybrids as Cardiovascular Drugs

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Abstract: The aim of this work was to develop lead pharmacodynamic hybrids, NO-sartans, possessing the characteristics of a typical AT_1-antagonist and of a "slow NO donor", by adding NO-donor side chains to losartan. These new compounds, **2a** and **2b**, displayed vasorelaxing effects, due to the release of NO, and antagonized the vasocontractile effects of angiotensin II, with potency values similar to that of losartan. In vivo, the antihypertensive effects of **2a** were similar to those of losartan and captopril.

The pharmacotherapy of hypertension involves several different classes of drugs; among these, the ones that have the renin−angiotensin system (RAS) as the target of their mechanism of action deserve special mention. ACE inhibitors belong to this group, seeing that they act by inhibiting the angiotensin converting enzyme (ACE), which is mainly responsible for the conversion of angiotensin I into angiotensin II. The latter is one of the most hypertensive substances in our organism: stimulation of the AT1 receptor by angiotensin II determines the release of aldosterone, which, by means of a reabsorption of Na^+ ions (and consequently of liquids) and a loss of K^+ ions, provokes hypertension. Furthermore, angiotensin II induces a direct vasoconstrictor action, which plays a significant role in hypertensive effects.

ACE is also involved in the mechanism of degradation of many other peptides, such as bradykinin. This peptide induces the release of endothelial nitric oxide (NO), a factor which plays a key role in the endogenous process of vasodilatation. The fact that ACE inhibitors save bradykinin also leads to a vasorelaxing effect, which contributes to the antihypertensive action that is mainly due to the lack of angiotensin II production.[1,2] Recent studies seem to indicate the possible existence of bradykinin-potentiating effects of ACE inhibitors, which are independent of the reduction of bradykinin hydrolysis; this mechanism was initially demonstrated by using bradykinin analogues which were assumed to be ACE-resistant.[3−7] This hypothesis is based on a "cross-talk" between ACE and bradykinin type 2 (B_2) receptors[8] (for example, forming a heterodimer), which leads to an up-regulation of the B_2 receptor,[5] probably accompanied by an up-regulation[9] and direct activation of bradykinin type 1 (B_1) receptors by ACE inhibitors.[10]

In view of the above-mentioned reasons, ACE inhibitors represent a first-choice class in hypertension therapy. Furthermore, they also reveal an implication at the cardiac level: angiotensin II inhibits muscular apoptosis, thus provoking ventricular hyperplasia, which might aggravate a preexistent cardiac failure.

However, ACE inhibitors also frequently present an adverse side-effect which is invalidating in social life and which drastically reduces the patient's compliance: this is coughing, which is the result of the ability of ACE inhibitors to preserve bradykinin, a kinin that stimulates the cough-center, from hydrolysis.[11]

In the past decade, research on drugs which can replace ACE inhibitors in hypertension therapy, without their collateral effects, has led to the discovery of sartans.[12] Drugs of this class are antagonists of angiotensin II at the AT1 receptor and block the action of angiotensin II in a potentially more complete way than ACE inhibitors; as is known, there are other enzymes, besides ACE, which are able to contribute to angiotensin II production.[13] Many comparative clinical trials have shown that sartans do not induce coughing because they do not prevent ACE from degrading bradykinin; in the past, they were employed when coughing was intolerable in a patient receiving therapy with ACE inhibitors, but nowadays, in view of the evidence presented, sartans can be viewed as a first-choice class of antihypertensive drugs.[14,15] However, sartans lack the enhancement of the NO-mediated vasorelaxing effect due to bradykinin preservation. The importance of NO lies not only in its vasorelaxing action, but also in its potent inhibition of platelet and neutrophil aggregation in the endothelium.[16,17]

The so-called "nitric oxide-releasing drugs" have their pioneers in nitrates and nitrites used in the treatment of angina pectoris[18,19] in view of their ability to release NO. On the basis of knowledge about nitric oxide properties, we have witnessed, in recent years, a proliferation of hybrid drugs in which a well-known molecule, with a certain pharmacodynamic pattern, has been integrated with an NO-donor group, thus conferring an improved pharmacological profile or a reduction of adverse effects. For example, much interest has been dedicated to NO-releasing nonsteroidal antiinflammatory drugs (NO-NSAIDs). Among these, NO-releasing aspirin has displayed increased antithrombotic properties in both in vitro and in vivo studies.[20−22] Furthermore, NO-NSAIDs may be beneficial in the early phases of myocardial infarction as a result of their NO-induced myocardial energy-sparing effect and the coronary vasodilatation that they induce, together with the well-known protective role played by NO during ischaemia.[23] In fact, NO-NSAIDs in general protect the myocardium during ischaemia reperfusion, by releasing NO; in particular, NCX-4016 significantly reduced infarct size in a rat model of myocardial infarction.[24−26] As regards other cardiovascular drugs, NO-releasing ACE inhibitors, NO-releasing calcium antagonists, and NO-releas-

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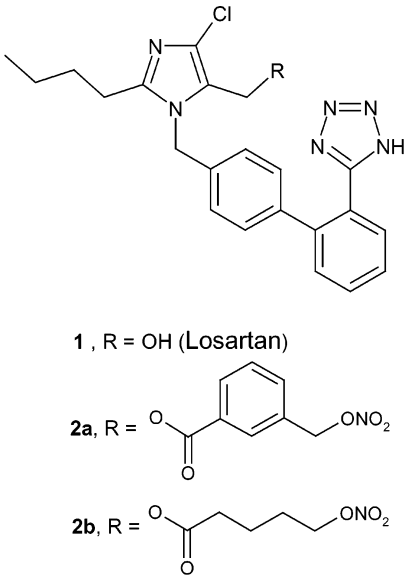


Figure 1. Structure of losartan (**1**) and general structure of the NO-releasing derivatives (**2a**,**b**) of losartan.

ing β-blocking agents have been synthesized, to improve the antihypertensive effects of the "native" drugs.[27−32]

The above-mentioned biopharmacological considerations, together with the appreciable results obtained in different classes of NO-releasing drugs, prompted us to develop a new class of drugs, by adding a NO-donor group to a sartan molecule. This kind of chemical manipulation was likely to give the "native" sartan an additional NO-mediated, but bradykinin-independent, vasorelaxing effect, thus generating the original class of NO-sartans, pharmacodynamic hybrids possessing the properties of a typical AT1 antagonist and of a "slow NO donor". The new compounds were expected to possess a pharmacodynamic profile very similar to that of ACE inhibitors, but without their bradykinin-mediated adverse effects.[33,34]

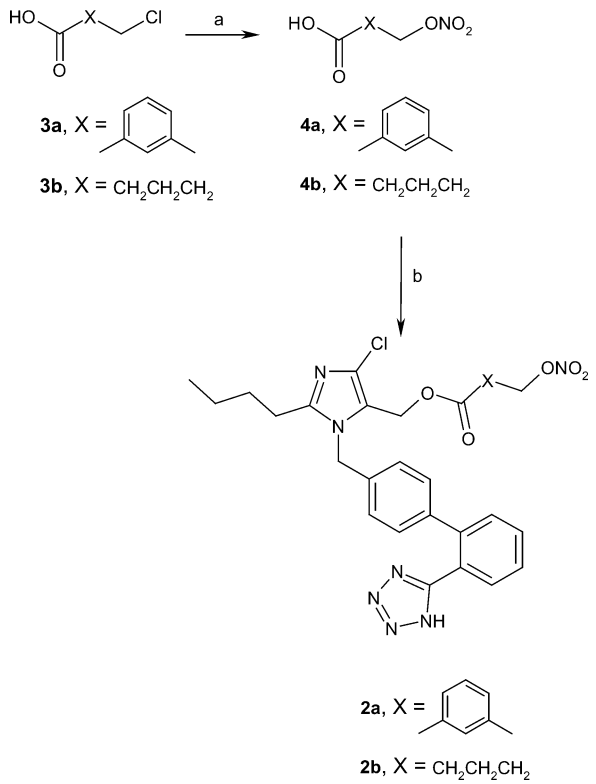
An examination of the structures of commercially available sartans showed that losartan (**1**) possesses both the high activity and the molecular features, i.e., the presence of an easily esterifiable alcohol group, suitable to provide a template for our purpose.

As regards the NO-donor moiety, the previously reported[35] *m*-nitrooxymethylbenzoate (**2a**, Figure 1) and the 5-nitrooxypentanoate (**2b**, Figure 1) were selected, based on the hypothesis of a different stability toward ester bond cleavage, depending on the aliphatic or aromatic nature of the compound.

The chemical pathway giving access to the final products **2a** and **2b** is reported in Scheme 1. *m*-(Chloromethyl)benzoic acid (**3a**) or 5-chloropentanoic acid (**3b**) were converted into their nitrooxy derivatives **4a** or **4b** by treatment with silver nitrate in acetonitrile at room temperature and in the dark. Condensation of the nitro esters (**4a**,**b**) with losartan in tetrahydrofuran, in the presence of *N,N*′-dicyclohexylcarbodiimide (DCC) and a catalytic amount of *N,N*-(dimethylamino)pyridine (DMAP), afforded the corresponding targets compounds **2a** and **2b**.

Pharmacological studies were carried out on the two "pharmacodynamic hybrids" **2a** and **2b**, by means of functional tests on vascular tissues, usually employed

Scheme 1[a]



[a] Key: (a) $AgNO_3$, CH_3CN, 1 h, rt; (b) losartan (**1**), DCC, THF, DMAP, 2 h, rt.

to determine NO-mediated responses[30] and AT1-antagonist activities.[36]

These two compounds induced full vasorelaxing effects (efficacy = 92 ± 6% and 95 ± 8%, for **2a** and **2b**, respectively; pIC_{50} = 5.55 ± 0.09 and 5.89 ± 0.14, for **2a** and **2b**, respectively) which were strongly inhibited by 1 μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), an inhibitor of guanylate cyclase, as to be expected in the case of an NO release effect (efficacy = 34 ± 3% and 23 ± 8%, for **2a** and **2b**, respectively; pIC_{50} values not calculable).

Also the AT1-antagonist properties of nitrooxy derivatives **2a**,**b** were investigated and compared with those of the reference AT1-antagonist losartan. The concentration−contractile response curve for angiotensin II was shifted parallel to the right by **2a** and **2b**, with K_b values of 6 nM and 16 nM, respectively. Under the same experimental conditions, losartan displayed a K_b value of 4 nM, substantially analogous to the value recorded for **2a** and slightly lower than that of **2b**.

Compound **2a** was selected in order to obtain further information, in an attempt to understand the pharmacokinetic steps leading to the pharmacodynamic features observed. To evaluate whether the vasorelaxing properties of **2a** are due to the release of NO from the whole molecule or from the side chain (**4a**), after its hydrolytic removal from losartan, the vasorelaxing effects of **4a** were evaluated by functional tests. Compound **4a** showed vasorelaxing effects lower than **2a**, both in efficacy and in potency (efficacy = 67 ± 12%; pIC_{50} = 4.66 ± 0.13). This first finding, which seems to indicate that the NO release from **2a** is more rapid, and thus that it precedes the possible hydrolysis of the ester link between losartan and **4a**, is confirmed by the

results obtained in the presence of eserin (3 μM, an inhibitor of esterases[37]). As expected, the presence of the esterase-inhibitor did not alter the vasorelaxing effects of **4a** (efficacy = 71 ± 6%; pIC_{50} = 4.64 ± 0.07); significantly, the effects of **2a** were not influenced by eserin, either (efficacy = 91 ± 3; pIC_{50} = 5.37 ± 0.05), confirming that the release of NO from this compound does not require previous hydrolytic removal of the side chain.

An analogous experimental strategy was employed to determine whether the AT1-antagonist properties of **2a** are due to the whole molecules or to losartan (after the hydrolytic removal of the side chains). As reported above, the AT1-antagonist potencies of losartan and of **2a** were quite indistinguishable, and in our opinion, this experimental evidence already tended to suggest that the antagonism was exerted by losartan itself, after the hydrolytic removal of the side chain, as desired for our purposes. The experimental confirmation of this hypothesis was provided by the use of eserin: in the presence of this esterase inhibitor, the antagonist potency of **2a** was dramatically lowered (K_b = 40 nM), while eserin did not influence the AT1-antagonist potency of losartan (K_b = 5 nM).

These initial functional data suggest that the main aim of this work, i.e., the creation of the original pharmacological class of NO-sartan AT1-antagonist/NO-donor hybrids, has been satisfactorily achieved. Furthermore, an "exploratory" in vivo protocol was carried out on **2a**, to obtain a preliminary indication about the possible profile of the antihypertensive action of a compound of this new pharmacological class, in comparison with that of an AT1-antagonist and an ACE inhibitor. Consequently, compound **2a**, losartan, and captopril were administered orally or subcutaneously to spontaneously hypertensive rats (SHR) for four weeks, recording the systolic blood pressure, by the "tail cuff" method.[38] The order of magnitude of the oral doses of captopril and losartan was selected on the basis of similar experimental protocols, described in the literature,[39] while **2a** was administered at a dose equimolar to that of losartan. For subcutaneous administration, the doses were reduced by one-half. In both these sets of experimental conditions, all compounds had practically equivalent effects, causing a significant reduction in the systolic pressure, which was lowered almost to the levels of reference normotensive animals (Figure 2). In our opinion, this result was of fundamental importance, since the ineffectiveness or a low effectiveness of **2a** was considered to be a critical factor, capable of invalidating the rational basis of our work.

In conclusion, the results of in vitro and in vivo studies confirm that these NO-sartans are actually pharmacodynamic hybrids possessing both the AT1-antagonist activity of sartans and the ancillary NO-releasing property of an NO donor. In addition, they seem to possess antihypertensive properties not inferior to those of sartans, and it is reasonable to conclude that this new class of drugs strengthens the action of the "native" sartans, integrating the satisfactory antihypertensive effects of the "native" drugs with all the other beneficial roles played by NO in the cardiovascular system.

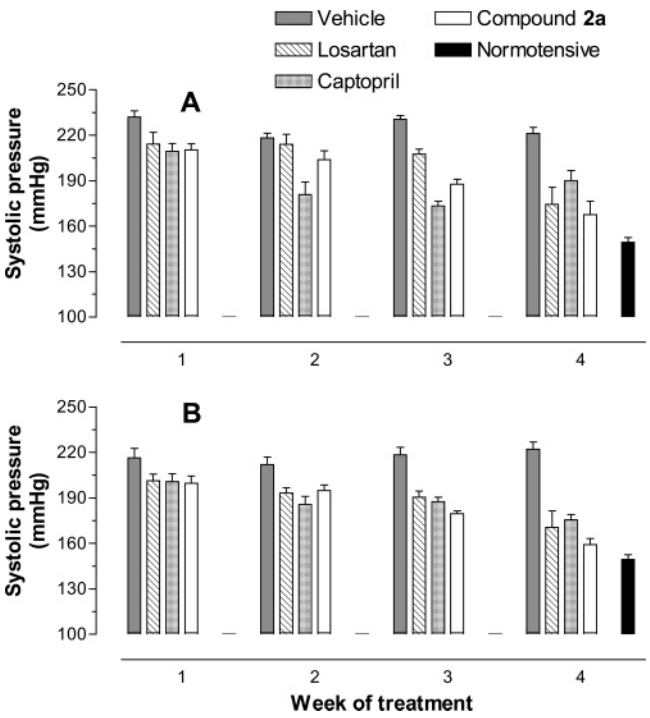


Figure 2. Histograms indicate the systolic blood pressure values recorded in the first four weeks of oral (A) or subcutaneous (B) pharmacological treatment with the vehicle, losartan, captopril, and compound **2a**. Systolic pressure values of reference normotensive animals are also shown. Vertical bars indicate the standard error.

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Supporting Information Available: Experimental procedures, ^{1}H NMR, and MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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