Design, Synthesis, and Biological Evaluation of Prazosin-Related Derivatives as Multipotent Compounds

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Received October 22, 2004

Abstract: To combine in the same molecule α_1-adrenoreceptor blocking and antioxidant properties, compounds **2**−**5** were designed and synthesized. All compounds were effective α_1-adrenoreceptor antagonists and were tested in both functional and binding assays. In addition, compounds **2** and **5** also displayed significant capacity to inhibit intracellular oxidative stress, whereas **3**−**5** exerted potent antiproliferative activity in lymph node carcinoma of prostate cells.

Heterogeneity of α_1-adrenoreceptors (α_1-ARs) has been revealed both on a molecular and pharmacological level. The latest picture of α_1-ARs shows at least three well characterized subtypes, i.e., $\alpha_{1A/1a}$, $\alpha_{1B/}\alpha_{1b}$, and $\alpha_{1D/}\alpha_{1d}$.[1] The effort to design agents selective for each of the three α_1-AR subtypes has been an active area of research because of the wide number of possible therapeutic applications. In addition to blood pressure reduction,[2] α_1-AR antagonists offer the advantage of a favorable effect on plasma lipoproteins and a low incidence of sexual dysfunction.[3] Recent data suggest that the activation of the α_{1A}-AR subtype may be responsible for ischemia-induced cardiac arrhythmia.[1,4] Therefore, α_{1A}-AR antagonists could be useful against this specific pathology. Furthermore, it has been shown that the α_{1A}-AR subtype is the predominant receptor involved in human prostate physiology, and consequently α_1-AR antagonists are effective drugs for the treatment of benign prostatic hyperplasia (BPH).[5] Recently, a role for the α_{1B}-AR subtype in the regulation of blood pressure has been advanced,[6] whereas a potential therapeutic use for the α_{1D}-AR subtype has not been firmly established yet although they may have a role in the control of blood pressure because of their involvement in the contraction of a variety of vessels.[7] Furthermore, the α_{1D}-AR is predominant in the detrusor muscle and is upregulated in the detrusor of obstructed rats.[8] This suggests a relevant role for this subtype also in the control of the symptoms associated with BPH.

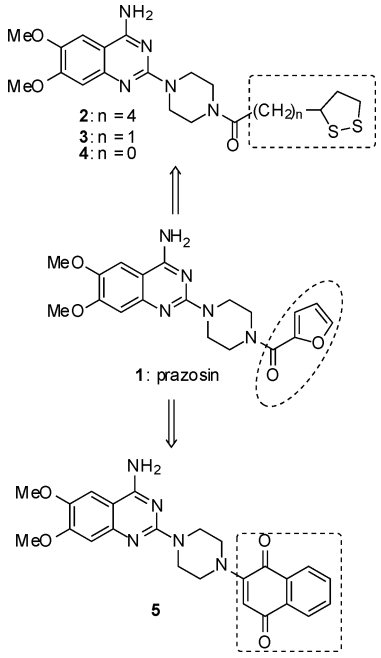

Figure 1. Design strategy for compounds **2**−**5**. The furoyl moiety of the prototype **1** was replaced by a 1,2-dithiolane-(alkyl)carbonyl (**2**−**4**) or a naphthoquinone (**5**) group.

Our research group has long being involved in designing new α_1-AR antagonists structurally related to prazosin (**1**),[9] the prototype of quinazoline-bearing compounds widely used as a pharmacological tool for α_1-AR subtypes characterization and as an effective drug in the management of hypertension. Together with **1**, its congeners, terazosin and doxazosin, offering a similar pharmacology with a longer duration of action, although not first line treatment, remain important options in the treatment of hypertension. Furthermore, **1** and its congeners together with tamsulosin represent the most used approach in treating lower urinary tract symptoms associated with or suggestive of BPH.[5]

With the aim of widening the biological profile of **1**-related compounds, further modifications of the lead compound **1** were accomplished to combine in the same molecule multiple biological activities. Thus, by replacing the furoyl moiety of **1** with the lipoyl fragment of lipoic acid (LA) or of its lower homologues or with 1,4-naphthoquinone, affording **2**−**5**, it was planned to achieve derivatives endowed with both α_1-AR antagonist and antioxidant properties. Our design strategy for compounds **2**−**5** is shown in Figure 1. Compounds **2** and **3** were synthesized to verify the importance, if any, on the biological profile of a longer spacer between carbonylpiperazine and 1,2-dithiolane rings. The choice of LA and homologues was dictated by the observation that LA is known as a universal antioxidant. The antioxidant activity of LA is attributed to its capacity to scavenge a number of free radicals in both membrane and aqueous domains, by chelating transition metals in biological systems, by preventing membrane lipid peroxidation and protein damage through the redox regeneration of endogenous antioxidants such as vitamin E (tocopherol),

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10.1021/jm049153d CCC: $30.25
Published on Web 12/17/2004

vitamin C (ascorbic acid), and notably gluthathione (GSH), thus maintaining an intracellular antioxidant balance.[10]

There is a growing interest in these natural antioxidants as a protective strategy against the pathologies associated to the oxidative stress, which can be broadly defined as an imbalance between oxidant production and cells' antioxidant capacity to prevent oxidative injuries. This phenomenon is imputable to high levels of reactive oxygen (ROS) and reactive nitric oxide species.

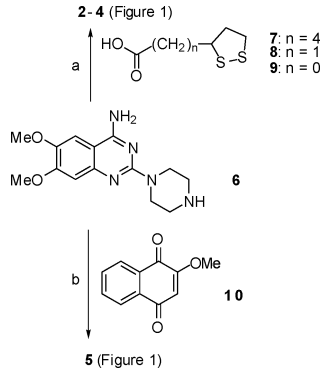
A variety of investigations performed in recent years have pointed out the noteworthy importance of the protective effects exerted by LA: (i) In various animal models, LA antioxidant properties would mediate antihypertensive effects and the prevention of insulin resistance, by normalizing superoxide anion production in aorta and preventing the decrease of glutathione peroxidase activity.[11] (ii) LA also ameliorates renal dysfunctions and tissue injuries caused by ischaemia/reperfusion by quenching radicals and restoring any antioxidant impairment.[12] (iii) LA supplementation decreases the urinary concentration of specific biomarkers of lipid peroxidation and increases the lag time of LDL lipoproteins oxidation.[13] This aspect is extremely interesting, since oxidative modifications of lipoproteins, and particularly of LDLs, appear to be one of the earliest phenomena involved in atherogenesis and development of atherosclerosis pathology, in addition to low levels of high-density lipoproteins (HDLs). Thus, antioxidant compounds, able to protect lipids from peroxidation, are of great value, to prevent and treat this pathology.[14]

We describe here the synthesis and the pharmacological profile of quinazolines **2**−**5** in functional and binding experiments at α_1-AR subtypes. In addition, antioxidant properties of **2**−**5** were evaluated by measuring the formation of intracellular ROS evoked by exposure of lymph node carcinoma of the prostate (LNCaP) cells to *tert*-butyl hydroperoxide (*t*-BuOOH), a compound used to induce oxidative stress. Finally, compounds **2**−**5** were tested as antiproliferative drugs because it has been reported that **1**-related derivatives, namely, terazosin and doxazosin, exert apoptotic activity in stroma smooth muscle and epithelial cell populations in prostate tissue.[15]

Synthesis of quinazolines **2**−**5** was accomplished by reaction of the known quinazolinylpiperazine **6**[16] with LA (**7**), 2-(1,2-dithiolan-3-yl)acetic acid (**8**),[17] 1,2-dithiolane-3-carboxylic acid (**9**),[18] or 2-methoxy-1,4-naphthoquinone (**10**), respectively (Scheme 1). No attempt was made to obtain the enantiomers of **2**−**4** because it was reported that stereochemistry is not relevant for the protective effect of LA against oxidative cell damage.[19]

Receptor subtype selectivity of compounds **2**−**5** was determined at α_1- and α_2-ARs on different isolated rat tissues. α_1-AR subtypes blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of prostatic vas deferens (α_{1A}) or thoracic aorta (α_{1D}) and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}), while α_2-AR blocking activity was determined by antagonism of the clonidine-induced depression of the twitch responses of the field-stimulated prostatic portion of rat vas deferens as previously described in detail.[9,20] The pharmacological profile of compounds **2**−**5** was further evaluated by radio-receptor binding assays using [^{3}H]prazosin to label cloned human α_1-ARs expressed in Chinese hamster ovary (CHO) cells as previously described.[9,21] The affinity for the three α_1-AR subtypes of compounds used in the present study was expressed as pK_b and pK_i values and is shown in Table 1.

Scheme 1. Synthetic Scheme for Quinazoline **2**−**5**[a]



[a] Reagents and conditions: (a) **7**, **8**, or **9**, DMF, EDCI·HCl, 0 °C to room temperature, 2 h, flash chromatography, 43%, 15%, or 41%; (b) **10**, EtOH, 70 °C, 7 days, flash chromatography, 13%.

Table 1. Affinity Constants, Expressed as pK_B or pA_2 (Isolated Tissues) or pK_i (CHO Cells) Values, at α_1-ARs on Isolated Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D}) and at Human Recombinant α_1-AR Subtypes (pK_i)

	pK_B or pA_2 [a]			pK_i [b]		
no.	α_{1A}	α_{1B}	α_{1D}	α_{1a}	α_{1b}	α_{1d}
1	8.98 ± 0.05[c]	9.01 ± 0.05[c]	9.01 ± 0.06[c]	9.23	9.39	9.65
2	7.19 ± 0.10	7.39 ± 0.12	7.92 ± 0.22	8.85	8.90	9.05
3	9.00 ± 0.11	8.99 ± 0.21	9.04 ± 0.06	9.54	9.51	9.41
4	7.73 ± 0.05	8.76 ± 0.20	9.06 ± 0.06	9.09	9.27	9.31
5	7.64 ± 0.04[c]	8.51 ± 0.10[c]	9.05 ± 0.05[c]	8.90	9.17	9.03
LA	< 5	< 5	< 5	nt[d]	nt[d]	nt[d]

[a] pK_B values ± SD (unless otherwise specified) were calculated at one concentration (in the range of 0.1−10 μM) according to van Rossum.[22] Each concentration was tested from four to five times. [b] Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation.[23] Each experiment was performed in triplicate. K_i values were from two to three experiments, which agreed within ±20%. [c] pA_2 ± SE were calculated from Schild plots,[24] constrained to slope of −1.0. All slopes were not significantly different from unity ($p > 0.05$). [d] nt, not tested.

Compounds **2**−**4** are characterized by the presence of the 1,2-dithiolane system, separated from the piperazine ring by a spacer of different length and just chain length modifications deeply influenced the pharmacological profile of the synthesized compounds. Derivative **2**, in which the piperazinylquinazoline nucleus of the prototype **1** was linked to LA molecule, showed a moderate affinity for all three α_1-ARs, without any relevant subtype selectivity. Compound **3**, in which the spacer length was decreased to one methylene, displayed a noteworthy potency enhancement toward all α_1-AR subtypes, resulting even more potent than **1** at both α_{1A}- and α_{1D}-ARs, without, however, any remarkable subtype selectivity. This aspect could suggest that the receptor might possess a definite binding area, probably the same lodging the furan ring of **1**, located in a precise region of the receptor, relative to the quinazoline binding-site: the 1,2-dithiolane system would interact with that receptor site only when it is at an appropriate distance from the piperazine ring. Derivative **4** is probably even more interesting: still displaying high potency toward the α_{1D}-AR subtype, it is, on the contrary, less

potent at α_{1A}- and α_{1B}-subtypes, resulting, therefore, about 20-fold more selective for the α_{1D}- relative to the α_{1A}-AR. As regard compound **5**, it displayed the same biological activity and selectivity profile of **4** at the different α_1-AR subtypes. Compounds **2**-**5**, like prototype **1**, were not active at α_2-ARs up to a concentration of 10 μM.

The results obtained in binding experiments for compounds **2**−**5** did not show the same affinity profile observed in functional assays. It can easily be seen that binding affinities of compound **3** are quantitatively in disagreement with pK_B values derived from functional experiments, whereas binding profiles of **2**, **4**, and **5** are not in agreement with functional data from both a qualitative and a quantitative point of view. As a matter of fact, **5**, in binding assays, showed an increase in affinity of more than 1 order of magnitude at the α_{1A}-AR relative to the α_{1D}, losing almost completely the relevant subtype selectivity registered in functional assays. There is no apparent explanation for the discrepancy noticed between binding and functional data, unless this difference is explained, among other possibilities, by admitting, as previously advanced,[25] that these compounds are inverse agonists, and hence their affinity is system-dependent. Another possible explanation might be found in the observation that receptor systems form homo- and heterodimers. For example, it has been demonstrated that the $GABA_BR1$ and $GABA_BR2$ receptor subtypes form heterodimers in vivo that are required for proper cell surface receptor localization and function.[26−29] Furthermore, in another study[30] it has been shown that κ and δ opioid receptors can form heterodimers with distinct ligand binding and functional properties, raising the possibility that heterodimerization may represent a more general mechanism to modulate GPCRs function. These observations of dimerization and, what may be even more intriguing, heterodimerization open a whole vista of possibilities for subtle changes in the pharmacology of GPCRs, which may be due to dimerized receptors. These new receptor entities may not always signal as either a monomer or as dimers. It derives that the presence of homodimeric or heterodimeric receptors may certainly have profound ramifications with regard to the interpretation of biological data and, as a consequence, to the validity of structure−activity relationships. Thus, the discrepancy often observed, like in the present case, between functional and binding affinities may not represent an anomaly, as in screening procedures a homogeneous population of cloned receptors is used, which can be organized differently than native receptors in functional tissues, and consequently their biological behavior may not be coincident. However, the discrepancy between functional and binding affinities observed in the present study may simply be accounted for by a different bioavailability of the compounds at the receptor level or by species differences in affinity.

Compounds **2**−**5** were designed and synthesized with the aim to associate vasorelaxant and antioxidant properties and verify whether they may have, like other **1**-related compunds,[15] antiproliferative activity.

The antiproliferative activity of LA, **1**−**6**, and **10** were determined by colorimetric MTS assay in LNCaP cells, the results being expressed as IC_{50} (concentration of compound resulting in 50% inhibition of cell proliferation). As reported in Table 2, treatment of LNCaP cells with LA, **2**, **6**, and **10** did not show modified cell proliferation. By contrast, the treatment of LNCaP cells with **1** (IC_{50} = 67.46 μM), **3** (IC_{50} = 52.62 μM), **4** (IC_{50} = 49.59 μM), and **5** (IC_{50} = 8.82 μM) produced a decrease of cell proliferation. In particular, compound **5** showed the highest antiproliferative activity.

Table 2. Effects on Cell Proliferation and ROS Formation in LNCaP Cells

no.	antiproliferative activity, IC_{50} (μM)[a,b]	antioxidant activity, IC_{50} (μM)[a,c]
LA	na[d]	54.86 ± 4.39
1	67.46 ± 0.20	na[d]
2	na[d]	83.53 ± 6.68
3	52.62 ± 3.90	na[d]
4	49.59 ± 2.39	na[d]
5	8.82 ± 0.10	2.63 ± 0.21
6	na[d]	na[d]
10	na[d]	na[d]

[a] The values are the mean ± SD of three independent experiments. [b] IC_{50} is the concentration of compound resulting in 50% inhibition of cell proliferation. The cell proliferation in LNCaP cells was determined by the MTS assay (as described in the Experimental Section), after 72 h of incubation with compounds (1−100 μM). [c] IC_{50} is the concentration of compound resulting in 50% inhibition of intracellular ROS formation. The intracellular ROS formation evoked by exposure of LNCaP cells to *t*-BuOOH was determined by a fluorescent probe (as described in the Experimental Section), after 24 h of incubation with compounds (1−100 μM). [d] na, not active at 100 μM.

The antioxidant activity of LA, **1**−**6**, and **10** against formation of ROS in LNCaP cells after treatment with *t*-BuOOH was then assessed, the results being expressed as IC_{50} (concentration of compound resulting in 50% inhibition of intracellular ROS formation). A range of concentrations of tested compounds that did not affect cell proliferation was used. As shown in Table 2, treatment of LNCaP cells with LA (IC_{50} = 54.86 μM), **2** (IC_{50} = 83.53 μM), and **5** (IC_{50} = 2.63 μM) showed a decrease of ROS formation while the treatment with **1**, **3**, **4**, **6**, and **10** did not modify the ROS formation. Remarkably, compound **5** showed the highest antioxidant activity.

Taken togheter, these results show that compounds **1** and **3**−**5** exerted antiproliferative effects, while LA, **2**, **6**, and **10** did not affect the cell proliferation. In addition, LA, **2**, and **5** (but not **1**, **3**, **4**, **6**, and **10)** were able to protect prostate cells against ROS formation evoked by oxidative stress. Thus, shortening the chain length separating the 1,2-dithiolane ring from the carbonyl function, as in **3** and **4**, produced a negative effect on the antioxidant properties as these compounds did not display a significant reduction of ROS up to a concentration of 100 μM. This finding suggests clearly that, at least in this series of compounds, antioxidant properties are retained by compounds that incorporate the LA structure but not that of its lower homologues. In contrast, compounds **3** and **4**, but not **2**, displayed an antiproliferative activity higher than that of prototype **1**. Interestingly, compound **5** showed both antiproliferative and antioxidant effects with relevant potency. However, based on the compounds evaluated, there does not appear to be a clear relationship between antioxidant and antiproliferative activity.

In conclusion, the present investigation has shown that it is possible to obtain multipotent drugs that are

able to display both a potent α_1-adrenoreceptor antagonism and the capacity to inhibit the oxidative stress and exert antiproliferative activity.

Acknowledgment. This work was supported by Grants from the University of Bologna and MIUR.

Supporting Information Available: Full experimental procedures and characterization data for all compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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JM049153D