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New Potential Uroselective NO-Donor α1-Antagonists

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A recent uroselective α1-adrenoceptor antagonist, REC15/2739, has been joined with nitrooxy and furoxan NO-donor moieties to give new NO-donor α1-antagonists. All the compounds studied proved to be potent and selective ligands of human cloned α1A-receptor subtype. Derivatives 6 and 7 were able to relax the prostatic portion of rat vas deferens contracted by (−)-noradrenaline because of both their α1A-antagonist and their NO-donor properties.

Introduction

Benign prostatic hyperplasia (BPH) is a widely diffused pathology in the aging male population. It consists of a progressive enlargement of the prostate that results in an obstruction of the proximal urethra.1 The hyperplastic prostate tissue contracts under sympathetic stimulation, principally mediated by α1-receptors. At present, native α1-receptors appear to exist as three subtypes encoded by three genes, α1A/α1A, α1B/α1B, α1D/α1D, where upper and lower case letters indicate subtypes from animal or human tissue and cloned subtypes, respectively.2 There is functional evidence for an additional α1-subtype (α1L) with a low affinity for prazosin.3 It may represent a different affinity state of α1A-adrenoceptors. In the human prostate, α1A-adrenoceptors are mainly present but the α1B-subtype also seems to play a role. Consequently a pharmacological approach used in the symptomatic treatment of BPH involves the employment of α1A-antagonists.3

Nitric oxide NO is an important biological messenger that elicits a surprisingly wide range of physiological effects on the cardiovascular system, the central and peripheral nervous systems, and the immune system.4 In the peripheral nervous system, it is the neurotransmitter at some nonadrenergic noncholinergic (NANC) effector junctions, and consequently, it is implicated in many genitourinary tract activities. In particular in the prostate, NO as a neurotransmitter and as a paracrine factor can modulate smooth muscular tone and secretory functions.3

On these bases, to develop our previous work on NO-donor α1-antagonists,6 we designed a series of "hybrid" drugs in which we joined the structure of REC15/2739 (1), a recent uroselective α1-adrenoceptor antagonist, discovered in Recordati S.p.A. laboratories,7 with nitrooxy and furoxan NO-donor moieties. In this paper, we report the synthesis, the structural characterization, and the pharmacological profile of novel compounds 5–7 and 10 tested for their α1-, α2-, and 5-HT1A-receptor affinities and for the α1A-adrenoceptor antagonism on rat vas deferens.

Results and Discussion

Scheme 1 briefly describes the standard procedure used for the preparation of derivatives of 1. The thiol-induced NO generation by the final products was indirectly evaluated by determining, through Griess reaction, the amount of nitrite ion formed by NO oxidation.6 The results expressed as % NO2− (mol/mol) are reported in Table 1. Nitrite production is strongly dependent on the medium, the concentration, and the nature of the thiol employed, and thus, it is only indicative of the NO production that might occur in a cellular environment. In addition, it does not give information about the NO-redox form(s) involved in the release. The ability to produce nitrite ion follows the series 7 > 6 > 5 = 10. In tissues and cells, thiol-dependent NO production and/or enzymatic activation has been proposed for nitrate. Enzymatic activation cannot be excluded for furoxans either.9

The affinities of the furoxan and nitrooxy derivatives, as well as those of the reference compound 1 for cloned α1-adrenoceptors, were evaluated in binding assays on membranes prepared from CHO cells (Chinese hamster ovary cells) expressing human α1A-, α1B-, α1D-subtypes. Competition assays were performed using [3H]prazosin to label the cloned receptors.10 Similarly, the affinities of the products for the human cloned 5HT1A-serotoninergic receptor were evaluated in membranes prepared from human HeLa cells expressing this receptor. [3H]-8-hydroxy-2-(di-n-propylamino)tetralin ([3H]-8-OH-DPAT) was used as radioligand in the competition binding experiments.10 The affinities of the products for native α2-adrenergic receptors were carried out in membranes of rat cerebral cortex with [3H]rauwolscine as radioligand.11 The results expressed as Ki are reported in Table 1.

Analysis of the data shows that the nitrooxy derivative 10 displays a very high affinity for the α1A-adrenoceptor subtype. Its Ki value is 3.6-fold lower than that of the reference 1. This product also shows a

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significantly improved $\alpha_{1A}$-selectivity (37-, 77-, and 870-fold relative to the $\alpha_{1D}$-subtype, clonidine $\alpha_{1A}$ receptor, and native $\alpha_2$-adrenergic receptors, respectively) in comparison with the reference (4-, 17-, and 98-fold, respectively). By contrast, $\alpha_{1D}$-selectivities relative to the $\alpha_{1D}$-subtype are similar in the two products (13- and 12-fold, respectively). As far as the furoxan derivatives are concerned, their affinity for $\alpha_2$-adrenoceptors, and rat cortex $\alpha_2$-adrenoceptors. $\alpha_2$-P2 values are the mean of 6-14 determinations. They were estimated at two concentrations for 5-7 ($3 \times 10^{-9}$ and $1 \times 10^{-8}$ M for 5, $3 \times 10^{-8}$ and $1 \times 10^{-7}$ M for 6 and 7; in the case of derivatives 6 and 7, when the NO relaxing effect was observed, we determined $\alpha_2$ values in the presence of $1 \mu$M ODO, which was added to the bath at least 10 min before the addition of the antagonist). Since $\alpha_2$ values obtained at both concentrations were similar, the antagonism was assumed to be competitive. For 1 and 10, we determined an apparent $\alpha_2$ value at one antagonist concentration ($3 \times 10^{-9}$ and $1 \times 10^{-8}$ M, respectively). $\alpha_2$ values were obtained from two to three experiments (each performed in triplicate), which correspond to within 20%. A solution of the appropriate compound (20 $\mu$L) in dimethyl sulfoxide was added to 1980 $\mu$L of a 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) and MeOH, containing 5 $\times 10^{-4}$ M L-cysteine. The final concentration of the compound was $10^{-4}$ M. After 1 h at 37 °C, 1 ml of the reaction mixture was treated with 250 $\mu$L of Griess reagent. No production of nitrite was observed in the absence of L-cysteine.

The functional $\alpha_2$ antagonist activity of the products was evaluated by antagonism of (-)-noradrenaline (NA) induced contractions of the prostatic portion of rat vas deferens in comparison with the antagonism of the reference 1. All the substances were able to shift the cumulative concentration–response curve of NA to the right in a concentration-dependent and reversible manner. In the case of derivative 5, which is a feeble NO donor, there was a parallel shift without any reduction of the maximal effect. The other two furoxan derivatives 6 and 7, which are more potent NO donors, behaved differently. In fact, the shift to the right of the concentration–response curve of NA was accompanied by a decrease in the maximum effect (see Figure 1, panels A and B). In the case of cyano derivative 7, the most potent NO donor, the decrease had already occurred at the first concentration tested. The maximal response was restored when the experiments were repeated in the presence of 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one (ODQ), a well-known inhibitor of the soluble guanilate cyclase (sGC). Simple NO-donor furoxans showed similar behavior when tested under the same experimental conditions (data not shown). Thus, this decrease is most likely due to the dilating properties of NO on the tissue mediated by sGC. For each compound, we calculated $\alpha_2$ values at the concentrations reported in the footnote of Table 1, using the equation $\alpha_2 = \log (CR - 1) - \log (B)$.

Table 1. Pharmacological Profile and Nitrite Formation of 5–7 and 10 and Reference

<table>
<thead>
<tr>
<th>Compd</th>
<th>$\alpha_2$ SE</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1D}$</th>
<th>$\alpha_2$</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1D}$</th>
<th>5-HT1A</th>
<th>3-HS1</th>
<th>5-HT1A</th>
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<tr>
<td>1</td>
<td>8.42 ± 0.10</td>
<td>0.34</td>
<td>3.9</td>
<td>1.5</td>
<td>33.3</td>
<td>5.9</td>
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</tr>
<tr>
<td>2</td>
<td>8.21 ± 0.06</td>
<td>0.16</td>
<td>4.7</td>
<td>4.2</td>
<td>53.5</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.97 ± 0.06</td>
<td>1.8</td>
<td>15.9</td>
<td>20.4</td>
<td>359.0</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.46 ± 0.07</td>
<td>0.73</td>
<td>6.5</td>
<td>3.9</td>
<td>81.1</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8.64 ± 0.06</td>
<td>0.94</td>
<td>1.2</td>
<td>3.5</td>
<td>81.8</td>
<td>7.2</td>
<td></td>
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</table>

a Functional $\alpha_2$-adrenoceptor antagonism on the prostatic portion of rat vas deferens ($\alpha_{1A}$) and receptor binding affinity for human cloned 5HT1A-serotonergic receptors, and rat cortex 5HT2A-receptors. $\alpha_2$-P2 values are the mean of 6-14 determinations. They were estimated at two concentrations for 5-7 ($3 \times 10^{-9}$ and $1 \times 10^{-8}$ M for 5, $3 \times 10^{-8}$ and $1 \times 10^{-7}$ M for 6 and 7; in the case of derivatives 6 and 7, when the NO relaxing effect was observed, we determined $\alpha_2$ values in the presence of $1 \mu$M ODO, which was added to the bath at least 10 min before the addition of the antagonist). Since $\alpha_2$ values obtained at both concentrations were similar, the antagonism was assumed to be competitive. For 1 and 10, we determined an apparent $\alpha_2$ value at one antagonist concentration ($3 \times 10^{-9}$ and $1 \times 10^{-8}$ M, respectively). $\alpha_2$ values were obtained from two to three experiments (each performed in triplicate), which correspond to within 20%. A solution of the appropriate compound (20 $\mu$L) in dimethyl sulfoxide was added to 1980 $\mu$L of a 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) and MeOH, containing 5 $\times 10^{-4}$ M L-cysteine. The final concentration of the compound was $10^{-4}$ M. After 1 h at 37 °C, 1 ml of the reaction mixture was treated with 250 $\mu$L of Griess reagent. No production of nitrite was observed in the absence of L-cysteine.
they display potent antagonist properties at the α1A-adrenoceptor subtype present in the rat vas deferens. Derivatives 6 and 7 are able to relax this tissue contracted by NA because of both their α1A-antagonist properties and their abilities to release NO under the experimental conditions adopted.

Since KT-1, a hybrid obtained by combination of prazosin and nitrooxy moieties, also retains (in vivo as well) cardiovascular effects similar to those of both nitrates and α1-adrenoceptor blocking agents, all the products described in the present work might be of interest for further in vivo studies on their potentialities in the treatment of BPH. This symbiotic approach could have advantages on the simultaneous administration of two single active drugs because the resulting hybrid should display a more balanced pharmacokinetic profile during the entire course of its action and, possibly, an increased activity.

**Experimental Section**

The results of elemental analyses of the new compounds are within ±0.4% of the theoretical values.

N-(3-(4-(2-Hydroxy)phenyl)piperazin-1-yl)propyl)-3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxamide (2). A 1 M Br2B solution (55 mL) was added dropwise to a stirred solution of I (3.13 g, 5.00 mmol) in dry CH2Cl2 (60 mL), and the mixture was refluxed under N2 for 4 h. The mixture was cooled to 5 °C, and a KHCO3 saturated solution (120 mL) was added dropwise. The two phases were stirred for 1 h until the solid formed was dissolved. Then they were separated. The aqueous phase was extracted with CH2Cl2, and the organic layers were collected, washed with brine, dried, and evaporated. The yellow solid obtained (2.43 g, 98%) was characterized by comparison with an authentic sample supplied by Recordati S. p. A. and used for the next synthesis without further purification.

N-(3-(4-(2-(1-Methylfuroxan-4-ylmethoxy)phenyl)piperazin-1-yl)propyl)-3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxamide Dihydrochloride (5). Compound 3 (0.77 g, 4.00 mmol) was added to a stirred suspension of KT-1 (1.00 g, 2.00 mmol) and K2CO3 (1.10 g, 8.00 mmol) in dry DMF (13 mL). After being stirred at room temperature for 24 h, the solution was poured into ice-cooled water and the solid formed was filtered, washed with ice-cooled water, dried, and purified by flash chromatography (elucent, CH2Cl2/MeOH, 9.5:0.5). The resulting product (0.70 g, 50%) was dissolved in dry MeOH, and HCl-saturated MeOH was added to the solution. The addition of dry ethyl ether gave a white precipitate that was filtered and dried under vacuum at 40 °C for 3 days, affording the title derivative: mp 145–148 °C (dec). Anal. (C34H35N5O6.H2Cl.H2O) C, H, N, Cl.

Conclusions

The reported compounds are potent and selective ligands of a human cloned α1A-receptor subtype, and expressed in their intact original tissue are utilized. It would seem that receptors may be organized differently under the two respective conditions, and consequently, their biological behavior may be different. Or more simply, a different bioavailability of the compounds at the receptor level may exist. In addition, radioreceptor binding measures simple displacement of a ligand from the binding site whereas the functional response involves multiple coordinated steps of a more complex system.

**Figure 1.**

(A) Effect of 6 on contraction to NA in rat vas deferens: control cumulative concentration–response curve (square) and 6 at 3 × 10^{-6} M (solid triangle), 1 × 10^{-7} M (solid diamond), and 1 × 10^{-7} M after ODO incubation (open diamond). (B) Effect of 7 on contraction to NA in rat vas deferens: control cumulative concentration–response curve (square) and 7 at 3 × 10^{-6} M (solid triangle), 3 × 10^{-6} M after ODO incubation (open triangle), 1 × 10^{-7} M (solid circle), and 1 × 10^{-7} M after ODO incubation (open circle). (C) Effect of 10 on contraction to NA in rat vas deferens: control cumulative concentration–response curve (square) and 10 at 1 × 10^{-6} M (solid triangle), 3 × 10^{-6} M (solid circle), and 3 × 10^{-8} M after ODO incubation (open circle).
g (57%) of the product as a pale-yellow solid: mp 77 °C (dec). Anal. (C_{34}H_{32}N_{6}O_{6}) C, H, N.

N-(3-(4-(2-(3-Bromopropoxy)phenyl)piperazin-1-yl)-propyl)-3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxamide (10). 1,3-Dibromopropane (8, 0.49 mL, 4.80 mmol) and 6 N NaOH (0.80 mL, 4.80 mmol) were added to a stirred solution of 2 (1.20 g, 2.40 mmol) in THF (15 mL). The solution was heated at 45 °C until the disappearance of starting material by TLC (24 h). The solution was then diluted with EtOAc, washed with water and brine, and then dried and evaporated. The crude residue was purified by column chromatography (eluent, EtOAc/PE/7 N NH3 in MeOH, 6:3.5:0.5), gave 0.25 g (41%) of a pale-yellow solid that was further purified by two crystallizations with 95% EtOH: mp (EtOH) 131–133 °C. Anal. (C_{33}H_{36}BrN_{3}O_{4}) C, H, N.

Acknowledgment. Financial support from the Italian MIUR is gratefully acknowledged.

Supporting Information Available: Detailed experimental procedures on the radioligand binding assays and functional studies of compounds 1, 5–7, and 10, including a description of the standard techniques and instrument used for the synthesis of the compounds, and their NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

References


