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New Potential Uroselective NO-Donor α_1-Antagonists

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A recent uroselective α_1-adrenoceptor antagonist, REC15/2739, has been joined with nitrooxy and furoxan NO-donor moieties to give new NO-donor α_1-antagonists. All the compounds studied proved to be potent and selective ligands of human cloned α_{1a}-receptor subtype. Derivatives **6** and **7** were able to relax the prostatic portion of rat vas deferens contracted by (−)-noradrenaline because of both their α_{1A}-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 sympathic stimulation, principally mediated by α_1-receptors. At present, native α_1-adrenoceptors appear to exist as three subtypes encoded by three genes, α_{1A}/α_{1a}, α_{1B}/α_{1b}, α_{1D}/α_{1d}, 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 α_1-subtype (α_{1L}) with a low affinity for prazosin.[3] It may represent a different affinity state of α_{1A}-adrenoceptors. In the human prostate, α_{1A}-adrenoceptors are mainly present but the α_{1B}-subtype also seems to play a role. Consequently a pharmacological approach used in the symptomatic treatment of BPH involves the employment of α_{1A}-antagonists.[3]

Nitric oxide NO$^\bullet$ 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) neuroeffector junctions, and consequently, it is implicated in many genitourinary tract activities. In particular in the prostate, NO$^\bullet$ as a neurotransmitter and as a paracrine factor can modulate smooth muscular tone and secretory functions.[5]

On these bases, to develop our previous work on NO-donor α_1-antagonists,[6] we designed a series of "hybrid" drugs in which we joined the structure of REC15/2739 (**1**), a recent uroselective α_1-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 α_1-, α_2-, and 5-HT$_{1A}$-receptor affinities and for the α_{1A}-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.[8] The results expressed as % NO_2^- (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 α_1-adrenoceptors, were evaluated in binding assays on membranes prepared from CHO cells (Chinese hamster ovary cells) expressing human α_{1a}-, α_{1b}-, α_{1d}-subtypes. Competition assays were performed using [^{3}H]prazosin to label the cloned receptors.[10] Similarly, the affinities of the products for the human cloned 5HT$_{1A}$-serotoninergic receptor were evaluated in membranes prepared from human HeLa cells expressing this receptor. [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]-8-OH-DPAT) was used as radioligand in the competition binding experiments.[10] The affinities of the products for native α_2-adrenergic receptors were carried out in membranes of rat cerebral cortex with [^{3}H]rauwolscine as radioligand.[11] The results expressed as K_i are reported in Table 1.

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

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Scheme 1

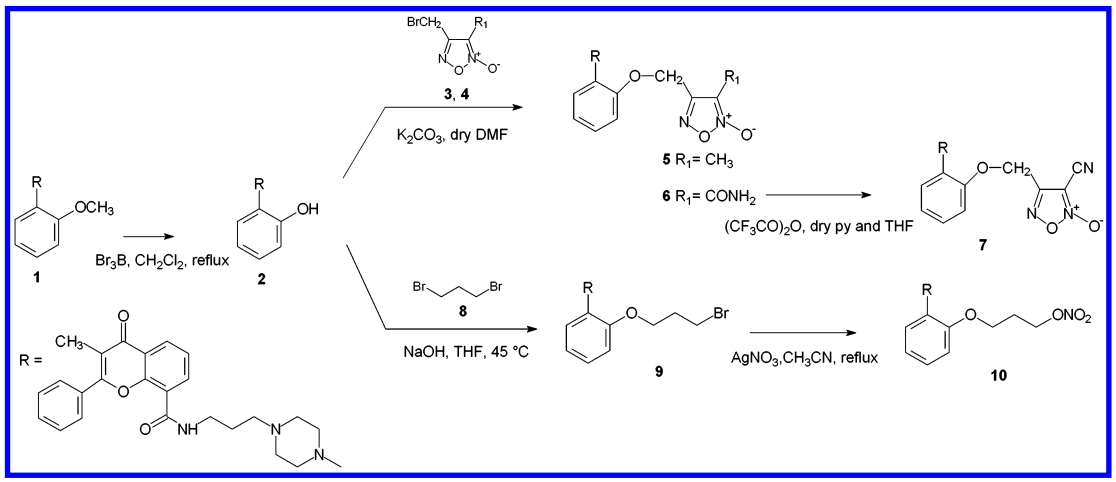

Table 1. Pharmacological Profile and Nitrite Formation of **5**−**7** and **10** and Reference **1**[a]

compd	$pA_2 \pm SE$[b] α_{1A}	inhibition of receptor binding, K_i[c] (nM) α_{1a}	α_{1b}	α_{1d}	α_2	5-HT_{1A}	% $NO_2^- \pm SE$[d] (mol/mol) Cys 5×
1	8.42 ± 0.10	0.34	3.9	1.5	33.3	5.9	
5	8.21 ± 0.06	0.16	4.7	4.2	53.5	1.6	1.11 ± 0.32
6	7.97 ± 0.06	1.8	15.9	20.4	359.0	1.2	5.28 ± 0.24
7	7.46 ± 0.07	0.73	6.5	3.9	81.1	2.2	32.3 ± 0.7
10	8.64 ± 0.06	0.094	1.2	3.5	81.8	7.2	<1

[a] Functional α_1-adrenoceptor antagonism on the prostatic portion of rat vas deferens (α_{1A}) and receptor binding affinity for human cloned α_1-adrenoceptor subtypes, $5HT_{1A}$-serotoninergic receptors, and rat cortex α_2-adrenoceptors. [b] pA_2 values are the mean of 6−14 determinations. They were estimated at two concentrations for **5**−**7** (3×10^{-9} and 1×10^{-8} M for **5**; 3×10^{-8} and 1×10^{-7} M for **6** and **7**; in the case of derivatives **6** and **7**, when the NO relaxing effect was observed, we determined pA_2 values in the presence of 1 μM ODQ, which was added to the bath at least 10 min before the addition of the antagonist). Since pA_2 values obtained at both concentrations were similar, the antagonism was assumed to be competitive. For **1** and **10**, we determined an apparent pA_2 value at one antagonist concentration (3×10^{-9} and 1×10^{-8} M, respectively). [c] K_i values were obtained from two to three experiments (each performed in triplicate), which correspond to within 20%. [d] A solution of the appropriate compound (20 μL) in dimethyl sulfoxide was added to 1980 μL of a 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) and MeOH, containing 5×10^{-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 μL of Griess reagent.[8] No production of nitrite was observed in the absence of L-cysteine.

significantly improved α_{1a}-selectivity (37-, 77-, and 870-fold relative to the α_{1d}- subtype, cloned $5HT_{1A}$ receptor, and native α_2-adrenergic receptors, respectively) in comparison with the reference (4-, 17-, and 98-fold, respectively). By contrast, α_{1a}-selectivities relative to the α_{1b}-subtype are similar in the two products (13- and 12-fold, respectively). As far as the furoxan derivatives are concerned, their affinity for α_{1a}-subtype is lower than that of **10** and, compared to reference, it follows the order **5** > **1** > **7** > **6**. The most active methyl derivative **5** also shows the best selectivity profile for α_{1a}-subtype.

The functional α_{1A} antagonistic 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*]quinoxalin-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 pA_2 values at the concentrations reported in the footnote of Table 1, using the equation $pA_2 = \log(CR - 1) - \log[B]$.[12] The average values are entered in Table 1. The nitrooxy derivative **10** behaved in quite a different manner. This product also induced a shift to the right of the concentration−response curve of NA but was accompanied at the high concentrations tested by a decrease of the maximal response that was not abolished by the presence of ODQ (Figure 1C). Consequently, this effect cannot be NO-dependent. This same decrease of the maximum effect with the increase of the concentration of the product is shown by reference **1** and its analogues in human prostate.[12] For these two products, we determined apparent pA_2 values at the lowest surmountable concentration giving a significant rightward shift of the concentration−response curves of the NA, using the above-reported equation. pA_2 values rank in the order **10** > **1** > **5** > **6** > **7**. Analysis of the results reveals that α_{1a} binding affinities (pK_i) of the compounds poorly correlate ($r^2 = 0.49$) with their antagonist potencies observed in NA-induced contractions of the prostatic portion of rat vas deference (pA_2). The discrepancy often observed between functional and binding affinities may be due to the different conditions of the receptors utilized in the assays. In binding procedures, homogenates of cell membranes bearing a homogeneous population of human cloned receptors are used, whereas in functional assays, rat native receptors

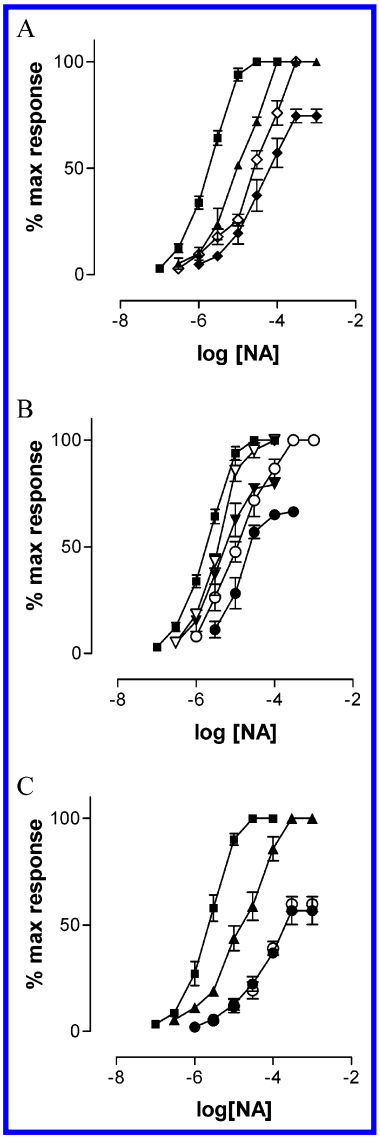

Figure 1. (A) Effect of **6** on contraction to NA in rat vas deferens: control cumulative concentration−response curve (square) and **6** at 3×10^{-8} M (solid triangle), 1×10^{-7} M (solid diamond), and 1×10^{-7} M after ODQ incubation (open diamond). (B) Effect of **7** on contraction to NA in rat vas deferens: control cumulative concentration−response curve (square) and **7** at 3×10^{-8} M (solid triangle), 3×10^{-8} M after ODQ incubation (open triangle), 1×10^{-7} M (solid circle), and 1×10^{-7} M after ODQ 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^{-8} M (solid triangle), 3×10^{-8} M (solid circle), and 3×10^{-8} M after ODQ incubation (open circle).

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.[13] 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.

Conclusions

The reported compounds are potent and selective ligands of a human cloned α_{1a}-receptor subtype, and they display potent antagonist properties at the α_{1A}-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 α_{1A}-antagonist properties and their abilities to release NO under the experimental conditions adopted.

Since KT-1,[14] 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 α_1-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-4*H*-chromene-8-carboxamide (2).** A 1 M Br_3B solution (55 mL) was added dropwise to a stirred solution of **1** (3.13 g, 5.00 mmol) in dry CH_2Cl_2 (60 mL), and the mixture was refluxed under N_2 for 4 h. The mixture was cooled to 5 °C, and a $KHCO_3$ 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 CH_2Cl_2, 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-(3-Methylfuroxan-4-ylmethoxy)phenyl)piperazin-1-yl)propyl)-3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxamide Dihydrochloride (5).** Compound **3** (0.77 g, 4.00 mmol) was added to a stirred suspension of **2** (1.00 g, 2.00 mmol) and K_2CO_3 (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 (eluent, CH_2Cl_2/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. ($C_{34}H_{35}N_5O_6 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

***N*-(3-(4-(2-(3-Carbamoylfuroxan-4-ylmethoxy)phenyl)-piperazin-1-yl)propyl)-3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxamide (6).** The title compound was prepared as described for **5** starting from **4** (0.88 g, 4.00 mmol). The obtained precipitate was purified by MPLC (eluent, CH_2Cl_2/7 N NH_3 in MeOH, 9.75:0.25). The resulting beige solid (0.80 g, 60%) was characterized as a free base: mp 189−195 °C (dec). Anal. ($C_{34}H_{34}N_6O_7 \cdot 1.5H_2O$) C, H, N.

***N*-(3-(4-(2-(3-Cyanofuroxan-4-ylmethoxy)phenyl)piperazin-1-yl)propyl)-3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxamide (7).** A solution of **6** (1.33 g, 2.00 mmol) in dry THF (75 mL) and dry pyridine (4.5 mL) was stirred under N_2 and cooled at 0 °C. To this solution trifluoroacetic anhydride (1.5 mL, 6.00 mmol) was added. After 15 min, the reaction was quenched by adding saturated $NaHCO_3$ solution (15 mL) and the THF was evaporated. The residue was extracted with EtOAc, and the combined organic layers were washed with water and brine and then dried and evaporated. The residue purified by MPLC (eluent, CH_2Cl_2/MeOH, 97.5:2.5) gave 0.71

g (57%) of the product as a pale-yellow solid: mp 77 °C (dec). Anal. ($C_{34}H_{32}N_6O_6 \cdot 0.5H_2O$) C, H, N.

***N*-(3-(4-(2-(3-Bromopropoxy)phenyl)piperazin-1-yl)-propyl)-3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxamide (9).** 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 material, purified by column chromatography (eluent, EtOAc/PE/7 N NH_3 in MeOH, 6:3.5:0.5), gave 0.80 g (54%) of the product as white powder that was crystallized from ethanol and dried at 40 °C under vacuum for 3 days: mp (EtOH) 131−133 °C. Anal. ($C_{33}H_{36}BrN_3O_4 \cdot 0.25H_2O$) C, H, N.

***N*-(3-(4-(2-(3-Nitrooxypropoxy)phenyl)piperazin-1-yl)-propyl)-3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxamide (10).** To a suspension of **9** (0.62 g, 1.00 mmol) in acetonitrile (10 mL), silver nitrate (0.34 g, 2.00 mmol) was added, and the reaction mixture was refluxed for 4 h. The silver bromine formed was filtered off, and the solvent was carefully evaporated. The residue was taken up in EtOAc, washed with water and brine, and then dried and evaporated. The crude residue was purified by column chromatography (eluent, EtOAc/PE/7 N NH_3 in MeOH, 6:3.5:0.5) and afforded 0.25 g (41%) of a pale-yellow solid that was further purified by two crystallizations with 95% EtOH: mp (EtOH) 139−142 °C (dec). Anal. ($C_{33}H_{36}N_4O_7$) C, H, N.

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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.

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