2-Aminopyridine Derivatives as Potential $\sigma_2$ Receptor Antagonists


$\sigma_2$ Receptor research is receiving increasing interest with regard to the potential of $\sigma_2$ proteins as targets for tumor therapy and diagnosis. Nevertheless, knowledge about the $\sigma_2$ receptor is far from conclusive. The paucity and modest affinity of known $\sigma_2$ antagonists represent one of the limitations to $\sigma_2$ receptor research. Previous studies of the high-affinity $\sigma_2$ agonist 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-n-propyl]piperazine 4 (PB28) suggested that a decrease in lipophilicity might lead to $\sigma_2$ ligands devoid of antiproliferative activity (potential $\sigma_2$ antagonists). With the aim of producing $\sigma_2$ receptor antagonists, we replaced the tetralin nucleus of compound 4 with a 2-aminopyridine moiety. A series of compounds with high affinity for both $\sigma$ subtypes and with no antiproliferative activity in various cells (mouse HT-22, human SK-N-SH, MCF-7wt, and MCF-7$\alpha_2$) were obtained. The effect on Ca$^{2+}$ mobilization was investigated for high-affinity compounds 18 and 4, which showed opposite effects. All of the data support the new 2-aminopyridines as high-affinity $\sigma$ ligands with $\sigma_2$ antagonist and $\sigma_1$ agonist activity, and, despite the lack of significant $\sigma_2$ versus $\sigma_1$ selectivity, these novel compounds may be better tools for $\sigma$ receptor research than the known low-affinity $\sigma_2$ antagonists.

Introduction

The two subtypes of sigma ($\sigma$) receptors, namely $\sigma_1$ and $\sigma_2$, represent potential and interesting targets for the diagnosis and therapy of different kinds of tumors and a number of central nervous system (CNS) diseases.[11,12] Since their discovery, impressive progress has been made in $\sigma$ receptor research, although several pieces of information are still missing for comprehensive knowledge about their mechanisms of action. Of the two subtypes, only $\sigma_1$ has been cloned from different sources.[3] Increasing evidence links this protein to neuroprotective and neuroregulatory functions and to CNS pathologies such as schizophrenia, depression, and Alzheimer’s and Parkinson’s diseases.[4–6] Recently, it has been shown that juvenile amyotrophic lateral sclerosis is caused by a mutation to the gene encoding the $\sigma_1$ receptor.[7] Diverse mechanisms of action have been proposed for the $\sigma_1$ protein, which has been assigned a chaperone function for cross-talk between the endoplasmic reticulum (ER) and the mitochondrion through Ca$^{2+}$ signaling, as well as a role in lipid compartmentalization.[8] The lesser-known $\sigma_2$ subtype has yet to be cloned. Attempts to isolate $\sigma_2$ receptors led to the hypothesis that they are related to histone proteins.[9,10] Nevertheless, later studies showed accumulation of $\sigma_2$ fluorescent ligands in diverse organelles except the nucleus.[11,12] Very recently, the $\sigma_2$ receptor has been identified as the progesterone receptor membrane component 1 (PGRMC1).[13] Increasing interest in $\sigma_2$ receptor research is mostly due to the diagnostic and therapeutic potentials that $\sigma_2$ ligands possess. This subtype is overexpressed in a number of cancer tissues, thus $\sigma_2$ radioligands and fluorescent ligands have been developed for the imaging of these proteins as biomarkers of tumors. Recently, a $\sigma_2$ receptor $^{18}$F-labeled radioligand entered a phase I clinical trial for application in positron emission tomography (PET) imaging of three kinds of tumors.[14] In addition, diverse fluorescent $\sigma_2$ receptor ligands have been employed to clarify the pathways activated by $\sigma_2$ proteins in tumor cells.[11,12] As for therapeutic potential, $\sigma_2$ ligands are under investigation for cancer treatment, as activation of $\sigma_2$ receptors leads to tumor cell death, and encouraging results have been shown for in vivo studies with $\sigma_2$ agonists for the treatment of tumors.[15,16]

On the other hand, it has been suggested that $\sigma_2$ antagonists mitigate many cocaine induced behaviors.[17] However, only a few $\sigma_2$ receptor antagonists are known in the literature, such as 1-(2-phenylethyl)piperidine (1, AC927, Figure 1),[18] 1-(2-phenylethyl)-4-(2-pyridyl)piperazine (2, UMB24, Figure 1),[17] and (±)-3x-tropanyl-2-(4-chlorophenoxy)butyrate (3, (±)-SM21, Figure 1).[19] In addition, these antagonists display low affinity for the $\sigma_2$ receptor (K, values $\geq$ 100 nm), so results obtained

[a] Dr. C. Abate, Prof. S. Ferorelli, Dr. M. Niso, Dr. C. Lovicario, Prof. Dr. R. Perrone, Prof. Dr. F. Berardi
Dipartimento Farmaco-Chimico
Università degli Studi di Bari ALDO MORO
Via Orabona 4, 70125 Bari (Italy)
E-mail: carmen.abate@uniba.it

[b] Dr. V. Infantino
Dipartimento di Chimica, Università della Basilicata
Viale dell’Ateneo Lucano 10, 85100 Potenza (Italy)

[c] Dr. P. Convertini
Dipartimento Farmaco-Biologico
Laboratorio di Biochimica e Biologia Molecolare
Università degli Studi di Bari ALDO MORO
Via Orabona 4, 70125 Bari (Italy)

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with these ligands are hardly conclusive. Therefore, there is a need for higher-affinity \( \alpha_2 \) antagonists as pharmacological tools to clarify the role of the \( \alpha_2 \) subtype in both in vitro and in vivo studies. In our previous work, we studied several analogues of 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-n-propyl]piperazine (4, PB28, Figure 1), which is one of the highest-affinity \( \alpha_2 \) receptor ligands known, displaying potent agonist activity in diverse tumor cells. According to this structure–affinity relationship (SAfR) studies, the N-cyclohexylpiperazine moiety was confirmed as an important feature for conferring high \( \alpha_2 \) receptor affinity. Moreover, the \( \alpha_2 \)-mediated antiproliferative activity of several analogues of compound 4 appeared to be correlated to lipophilicity. In fact, less lipophilic compounds do not exert antiproliferative activity and may therefore have an antagonist effect at the \( \alpha_2 \) receptor. Keeping this hypothesis in mind, we developed a series of N-cyclohexylpiperazines linked to a less lipophilic nucleus than tetralin with the aim of producing high-affinity \( \alpha_2 \) receptor antagonists. A tetrahydro-1,8-naphthyridine nucleus was used, in spite of the tetralin, to keep the bicyclic structure while the lipophilicity of the ligands was decreased. The corresponding monocyclic structure was also investigated, and a series of N-cyclohexylpiperazine derivatives in which the tetralin was replaced by the N-(pyridin-2-yl)ethylamino moiety was obtained. Both in the bicycle-bearing and monocyte-bearing cyclohexylpiperazine derivatives, the alkyl chain length was varied from three to five methylene moieties. Also, the effect of the insertion of an oxygen atom was investigated both in the bicyclic and monocyclic rings, generating the pyrido-oxazine and the 4-methoxy-2-amino pyridine moieties, respectively. The nitrogen atom adjacent to the pyridine was made into an amide to provide diverse electron lone pair availabilities.

**Results and Discussion**

**Chemistry**

The syntheses of final compounds 12–15, 18, 19, 22, 23, 26–29, 35, and 36 are depicted in Schemes 1–3. The preparation of final compounds 12–15, 18, and 19 is depicted in Scheme 1. 2-Amino-3-(bromomethyl)pyridine hydrobromide (5), obtained by boration of 2-amino-3-hydroxymethylpyridine, was reacted with dimethylmalonate and NaOCH\(_3\) to afford intermediate 6. Ester hydrolysis, followed by decarboxylation, provided key intermediate 3,4-dihydro-1,8-naphthyridin-2-(1H)-one (7). N-cyclohexylpiperazine moieties 8–10 were prepared by alkylation of N-cyclohexylpiperazine with 1-bromo-3-chloro-propane to afford intermediate 8, and by acylation with 4-chlorobutanoyl chloride and 5-chloro pentanoyl chloride to afford, respectively, intermediates 9 and 10. Treatment of compound 7, as well as of the commercially available 2H-pyrido[3,2-b]-1,4-oxazine-3-(4H)-one 11, with NaH and chloropropylperazine 8 afforded final compounds 12 and 13, respectively. These amide compounds were reduced with borane–tetrahydrofuran (BH\(_3\)·THF) complex to afford final compounds 14 and 15, respectively. Treatment of compound 7 with NaH and intermediates 9 or 10 furnished intermediate 16 or 17 which, upon reduction with BH\(_3\)·THF complex, provided final compounds 18 and 19 (Scheme 1).

The preparation of final compounds 22, 23, and 26–29 is depicted in Scheme 2. Acetamides 20 and 21, respectively obtained by acetylation of 2-amino pyridine and 4-methoxy-2-amino pyridine, were reacted with intermediate 8 in the presence of NaH to afford compounds 22 and 23, respectively. Intermediate 20 was reacted with chloroalkylpiperazine derivatives 9 and 10 to afford compounds 24 and 25, respectively. Reduction with BH\(_3\)·THF complex of the amide functionalities of compounds 22–25 provided final amine compounds 26–29.

The synthesis of final compounds 35 and 36 is depicted in Scheme 3. Key intermediate pyridine-1-oxide derivatives 30 and 31 were commercially available. However, the latter was synthesized through a previously reported synthesis starting from 2-chloro-4-nitropyridine-1-oxide via 2-chloro-4-methoxy pyridine. Compounds 30 and 31 underwent nucleophilic
substitution with 3-(4-cyclohexylpiperazin-1-yl)propanamine\(^{[32]}\) 32 to afford intermediates 33 and 34, which were converted with PCl\(_3\) to the final amine compounds 35 and 36, respectively.

All of the final amine compounds were converted into their hydrochloride or oxalate salts with gaseous HCl or oxalic acid, respectively, in anhydrous diethyl ether. Physical properties of these salts are listed in the Table of Physical Properties of Novel Compounds in the Supporting Information, along with the values of the calculated logarithm of distribution coefficient (ClogD) for the corresponding free bases.\(^{[33]}\)

As 2-aminopyridine is endowed with fluorescence properties, the fluorescence spectra of all compounds were recorded to evaluate whether fluorescence of these new compounds could be exploited in biological assays. Fluorescence spectra of final compounds as free bases (at 10\(^{-5}\)–10\(^{-6}\) M concentrations) were recorded in organic solvents (CHCl\(_3\), EtOH) and in aqueous buffer (pH 7.4), but the quantum yields (calculated using 2-aminopyridine as the reference compound) were too low for imaging purposes, so the fluorescence properties of these molecules were no longer considered.

Radioligand binding and \(\alpha_1\) and \(\alpha_2\) receptor affinities

Results from binding assays are expressed as inhibition constants (\(K_i\) values) in Table 1. Although none of the newly synthesized 2-aminopyridine derivatives displayed sub-nanomolar binding affinities similar to that of 4 at both \(\alpha\) receptors, appreciable affinity values were reached. No selectivity between the two \(\alpha\) subtypes was obtained, and most of the new compounds displayed a slight preference for the \(\alpha_1\) receptor (14, 15, 19, 26, 27, 35, and 36). On the other hand, compounds 12, 18, and 22 displayed slight \(\alpha_2\) selectivity, with only 12 and 18 characterized by appreciable \(\alpha\) affinity.

Binding values displayed by bicyclic 1,8-naphthyridine compounds (14, 18, and 19) and by corresponding open monocyclic 2-aminopyridine analogues (26, 28, and 29) were similar (\(K_i\): 2.06–5.66 nM for \(\alpha_1\); \(K_i\): 1.64–4.6 nM for \(\alpha_2\)), demonstrating that a decrease in conformational freedom does not affect the affinity at \(\alpha\) receptors. In addition, no significant effect of chain

![Scheme 2](image1.png)

Scheme 2. Synthesis of final monocyclic 2-aminopyridine derivatives. Reagents and conditions: a) NaH, DMF, RT, 48 h; b) BH\(_3\)·THF, THF, reflux, 3 h.

![Scheme 3](image2.png)

Scheme 3. Synthesis of final monocyclic 2-aminopyridine derivatives. Reagents and conditions: a) Et\(_3\)N, n-butanol, 120 °C, 20 h; b) PCl\(_3\), CHCl\(_3\), 80 °C, 1 h.

**Table 1. Binding data of final 2-aminopyridine derivatives.**

<table>
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<th>Compd</th>
<th>Z</th>
<th>n</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>X</th>
<th>Y</th>
<th>(K_i) [nM](^{[a]})</th>
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<td>O</td>
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<td>A</td>
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<td>O</td>
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<td>2.06 ± 0.71</td>
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<td>353 ± 14</td>
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<td>COCH(_3)</td>
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<td>4</td>
<td>H</td>
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<td>5</td>
<td>H</td>
<td>CH(_2)CH(_3)</td>
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\(\alpha_1\) and \(\alpha_2\) receptor affinities. 

(a) Values are the means ± SEM of \(n \geq 2\) separate experiments.
length (3–5 methylene groups) on $\alpha$ receptor affinity was recorded. The three-methylene chain analogues were further investigated both in the bicyclic and in the monocyclic series. In the latter series, the insertion of a methoxy group in the 4-position of the pyridine (compound 27) left affinity at both $\alpha$ subtypes almost unchanged ($K_i$ values of 7.68 and 30.1 nm for $\alpha_1$ and $\alpha_2$, respectively). The importance of the ethyl group on the 2-aminoypyridine moiety was investigated with pyridine and 4-methoxyypyridine analogues. Elimination of the ethyl residue resulted in a two- to fivefold decrease in affinity at both $\alpha$ subtypes (comparison of 35 with 26 and 36 with 27). Replacement of the ethyl with an acetyl group (compounds 22 and 23) in both pyridine and 4-methoxyypyridine analogues led to a dramatic decrease in affinity at the $\alpha_1$ receptors ($K_i = 535–402$ nm for the $\alpha_1$ receptor; $K_i = 246–309$ nm for the $\alpha_2$ receptor). This evidence suggests that a certain availability of the electron lone pair in the 2-aminoypyridine portion is important for a high-affinity interaction with $\alpha$ receptors. In addition, the small decrease in $\alpha$ binding that was recorded for secondary amines 35 and 36 suggests that, besides the lone pair availability, a certain bulk is useful for binding $\alpha$ proteins.

To study the effect of decreasing lone pair availability in the bicyclic series as well, 1,8-naphthyridin-2-one derivative 12 was produced. Apparently, the electron lone pair availability in the 2-aminoypyridine moiety is not a strict requirement for the interaction of bicyclic derivatives with $\alpha_1$ receptors. In fact, 12 showed an insignificant decrease in $\alpha_2$ affinity ($K_i = 16.1$ nm) relative to the non-amidic counterpart 14 ($K_i = 9.87$ nm). On the other hand, 12 showed a 30-fold lower $\alpha_1$ receptor affinity than 14. The bicyclic series was extended with the synthesis of two isosteres in which the 1,8-naphthyridine nucleus was replaced by a pyrido-oxazine in derivative 15 and by a pyrido-oxazinone in 13. Insertion of an oxygen atom (15) did not lead to pharmacodynamic changes at $\alpha$ receptors relative to 1,8-naphthyridine derivative 14. Presence of an amidic functionality (13) led to a decrease in $\alpha_2$ receptor affinity but not as significant as the decrease recorded for 1,8-naphthyridin-2-one 12. Conversely, no difference was recorded at the $\alpha_2$ receptor between the amidic (13) and non-amidic (15) derivatives, as in the 1,8-naphthyridine series. Overall, the bicyclic amide derivatives (12 and 13) did not show the same decrease in affinity as the monocyclic derivatives (22 and 23), particularly at $\alpha_2$ receptors, suggesting the hypothesis that diverse binding modes for the bicyclic and monocyclic series may be possible.

### Functional assays: antiproliferative activity in human neuroblastoma, human breast cancer, and mouse hippocampal cells

The antiproliferative activities of compound 4 and the newly synthesized compounds are reported as $EC_{50}$ values in Table 2. Four cell lines, HT-22 hippocampal mouse cells, SK-N-SH human neuroblastoma cells, and MCF-7 wild-type human breast adenocarcinoma cells (MCF-7wt) and MCF-7 cells transfected with $\alpha_1$ receptor (MCF-7$\alpha_1$) were selected for activity assays. As previously reported, the SK-N-SH cell line proved to be a good model for the evaluation of $\alpha_1$ receptor-mediated antiproliferative activity, as $\alpha_1$ receptors were reported to be present in a low affinity state. HT-22 cells have been used as a model for $\alpha_1$ receptor-mediated neuroprotection. Scatchard analysis, which we performed on HT-22 cells to determine the content of both $\alpha$ subtypes, surprisingly revealed a low $\alpha_1$ receptor density ($B_{\text{max}} = 0.169$ pmol mg$^{-1}$ of protein), and a sevenfold higher $\alpha_2$ receptor content ($B_{\text{max}} = 1.23$ pmol mg$^{-1}$ of protein, Figure 2). Therefore, this cell line was also mainly used for evaluation of $\alpha_2$ receptor-mediated action. To have a cell line
line expressing the $\alpha_1$ receptor with an appreciable density, transfection of MCF-7wt cells with the $\alpha_1$ receptor gene was conducted according to the literature.\(^{[23]}\) The density of both subtypes was determined before transfection, and the increase in $\alpha_1$ receptor content in the newly created MCF-7$\alpha_1$ cell line was evaluated after transfection (Figure 3). Therefore, MCF-7$\alpha_1$ was mainly used for the evaluation of $\alpha_1$-mediated action, whereas MCF-7wt was used for evaluation of $\alpha_2$ receptor-mediated action. Reference compound 4, which was previously defined as a $\alpha_2$ receptor agonist and a $\alpha_1$ receptor antagonist,\(^{[22,23]}\) was studied in the four cell lines where it showed antiproliferative activity (Table 2), as expected for a $\alpha_2$ receptor agonist and a $\alpha_1$ receptor antagonist. On the other hand, all of the new 2-aminopyridine derivatives did not display antiproliferative activity, with EC\(_{50}\) values $> 100 \mu M$ in all of the cell lines studied. Although the involvement of other proteins in the overall action of these $\alpha$ receptor ligands cannot be ruled out, these data are in agreement with previous results obtained with $\alpha_1$ ligands less lipophilic than 4, leading to the hypothesis that lower lipophilicity values lead to lower $\alpha_1$-mediated antiproliferative activity.\(^{[20]}\) In addition, compound 18, which represents the highest $\alpha_1$ affinity compound among the novel 2-aminopyridines, was further investigated in an antiproliferative assay in co-administration with 4. At the concentration used, 18 partially reversed the antiproliferative effect exerted by compound 4 in MCF-7wt cells (see the figure in the Supporting Information), showing that 18 is able to antagonize the $\alpha_2$-mediated antiproliferative action exerted by the $\alpha_2$ receptor agonist 4.

### $\alpha$ Receptor effect on intracellular $\text{Ca}^{2+}$ mobilization

Effects on intracellular $\text{Ca}^{2+}$ mobilization were evaluated for representative 2-aminopyridine 18 and for reference compound 4 with the aim of understanding whether the opposite effect exerted on cell proliferation corresponds to differences in intracellular $\text{Ca}^{2+}$ mobilization. Therefore, bradykinin-triggered $\text{Ca}^{2+}$ response was measured for 4, 18, and the prototypical $\alpha_1$ agonist (+)-pentazocine ([(+)-2S-(2α,6α,11R)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol] in SK-N-SH, MCF-7wt, and MCF-7$\alpha_1$ cells (Figure 4); none of the ligands affected intracellular $\text{Ca}^{2+}$ concentration when administered alone.

Although 4 and 18 do not display $\alpha$ selectivity, results obtained through experiments in the cell lines selected should allow retrieval of compound effects on each $\alpha$ subtype: SK-N-SH cells express both $\alpha$ receptors, with $\alpha_1$ subtype in a low affinity state; MCF-7$\alpha_1$ cells overexpress $\alpha_1$ receptors; MCF-7wt cells overexpress $\alpha_2$ receptors. As previously reported, the selective $\alpha_1$ agonist (+)-pentazocine increased bradykinin-induced $\text{Ca}^{2+}$ mobilization in SK-N-SH and MCF-7$\alpha_1$ cells, whereas no effect was exerted in MCF-7wt cells where the $\alpha_1$ density is too low (Figure 3A).\(^{[35,36]}\) Compound 4, as previously shown with carbachol in SK-N-SH cells,\(^{[37]}\) inhibited bradykinin-triggered $\text{Ca}^{2+}$ response in all of the cell lines studied, whereas it did not exert any effect in LoVo colon adenocarcinoma cells in which none of the $\alpha$ subtypes were detected through Scatchard analysis.\(^{[38]}\) These results support the idea that effects exerted by compound 4 in bradykinin-triggered $\text{Ca}^{2+}$ response are $\alpha$-mediated and suggest that agonist activity at $\alpha_2$ (from SK-N-SH and MCF-7wt) and antagonist activity at $\alpha_1$ (from MCF-7$\alpha_1$) decrease such response. On the other hand, 2-aminopyridine derivative 18, increased bradykinin-triggered $\text{Ca}^{2+}$ response in SK-N-SH and MCF-7$\alpha_1$ cells, similar to (+)-pentazocine, implying agonist activity at the $\alpha_1$ receptor. No effect was exerted by 18 in MCF-7wt cells, where the action may be mediated by the $\alpha_2$ receptor.

Together, these data show that compounds 4 and 18 have opposite behavior and suggest that while agonist activity at

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**Figure 3.** Saturation analysis of A) $\alpha_1$ ($K_\text{d} = 2.97 \text{ nM}$, $B_{\text{max}} = 0.172 \text{ pmol (mg protein)}^{-1}$) and B) $\alpha_2$ ($K_\text{d} = 9.3 \text{ nM}$, $B_{\text{max}} = 0.323 \text{ pmol (mg protein)}^{-1}$) receptors in membrane preparations from MCF-7wt cells, and saturation analysis of C) $\alpha_1$ ($K_\text{d} = 7.6 \text{ nM}$, $B_{\text{max}} = 3.45 \text{ pmol (mg protein)}^{-1}$) in membrane preparations from MCF-7$\alpha_1$ cells: total binding; nonspecific binding; specific binding.
the $\alpha_2$ receptor decreases bradykinin-induced $Ca^{2+}$ response, antagonist activity may not alter such response. Therefore, compound 18 may be proposed as a $\alpha_2$ receptor antagonist and a $\alpha_1$ receptor agonist.

Conclusions

A certain number of $\alpha_2$ receptor ligands with considerable $\alpha_2$ versus $\alpha_1$ selectivities are known [26, 32, 38] but no selective and high-affinity $\alpha_2$ antagonists has been reported yet, thus there is a need for $\alpha_2$ antagonists as tools for $\alpha$ receptor research. Therefore, with the aim of compensating for the paucity of $\alpha_2$ antagonists, we based the development of $\alpha_2$ ligands with potential antagonist activity on previous studies. Less lipophilic analogues than $\alpha_2$ receptor agonist 4 were obtained by replacement of the tetralin nucleus with a 2-aminopyridine moiety. Compounds with high affinity for both $\alpha_1$ receptor subtypes were obtained, and most were characterized by ClogD values within the optimal range for entry into cells, as demonstrated for $\alpha_2$ PET radiotracers by other authors. [38] None of the new compounds displayed antiproliferative activity in the four cell lines selected (mouse HT-22 and human SK-N-SH, MCF-7wt, and MCF-7$\alpha_1$ cells), in contrast with lead compound 4, which consistently showed micromolar antiproliferative activity. The highest-affinity $\alpha_2$ ligand 18 was co-administered with 4 in MCF-7wt cells and partially decreased the antiproliferative effect exerted by 4. To investigate whether the lack of antiproliferative action of the newly synthesized compounds corresponds to a different effect on $Ca^{2+}$ mobilization, representative compounds 18 and 4 were evaluated in three tumor cell lines with diverse $\alpha$ subtype content. Actually, compound 18 displayed an opposite effect than compound 4 on bradykinin-induced $Ca^{2+}$ response. All of the results together suggest a $\alpha_2$ receptor antagonist and a $\alpha_1$ receptor agonist activity for compound 18. In conclusion, despite the lack of $\alpha_2$ versus $\alpha_1$ selectivities, these new compounds may represent better tools for $\alpha$ receptor research than the low affinity and poorly selective $\alpha_2$ receptor antagonists known.

Experimental Section

Chemistry

Both column chromatography and flash column chromatography were performed with 60 Å pore size silica gel as the stationary phase (1:30 w/w, 63–200 μm particle size from ICN and 1:15 w/w, 15–40 μm particle size from Merck, respectively). Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Purity of tested compounds was established by combustion analysis, confirming purity $\geq$98%. Elemental analyses (C, H, N) were performed on an Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of theoretical values. $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded on a Mercury Varian spectrometer using CDCl$_3$ and CH$_3$OD, respectively, as solvent. The following data were reported: chemical shift (δ) in ppm, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integration, and coupling constant(s) in Hertz. Recording of mass spectra was done on an Agilent 6890-5973 MSD gas chromatograph/mass spectrometer and on an Agilent 1100 series LC–MS trap system VL mass spectrometer; only significant m/z peaks, with their percentage of relative intensity in parentheses, are reported. Chemicals were from Sigma–Aldrich and Alfa Aesar and were used without any further purification.

General procedure for the synthesis of compounds 12, 13, 16, 17, and 22–25: Nah (1.2 mmol, 0.29 g, 60% w/w) was added to a stirred solution of the corresponding amide compound (7, 11, 20, or 21; 1.0 mmol) in dry DMF (10 mL) under $N_2$. After 1 h, a solution of the appropriate chloride (1.0 mmol) in DMF was added to the suspension under $N_2$. The reaction mixture was stirred at room temperature for 48 h. Then, water was added to the reaction mixture, and the organic layer was separated. The aqueous phase was extracted with EtOAc (3 × 10 mL). The collected organic layers were dried over Na$_2$SO$_4$ and evaporated under reduced pressure to afford a crude residue, which was purified as reported below for each target compound.

1-(3-[4-Cyclohexylpiperazin-1-yl]propyl)-3,4-dihydro-1,8-naphthyridin-2(1H)-one (12): The brown semi-solid was purified by column chromatography with CH$_3$Cl/CH$_2$OH (98:2) as eluent to achieve the title compound as a yellow oil (0.125 g, 35%): $^1$H NMR δ = 1.06–1.38 [m, 5 H, cyclohexyl NCH(CH$_2$)$_2$], 1.58–1.70 [m, 1 H, cyclohexyl], 1.72-2.05 [m, 3 H, CHN, J = 7.55 Hz, CONCH$_2$CH$_2$N], 2.30–2.48 [m, 4 t, 3 H, CHN, $J = 7.55$ Hz, CONCH$_2$CH$_2$N], 2.54–2.80 [m, 10 H, piperase and CH$_2$CH$_2$CO], 2.86 (t, 2 H, $J = 7.9$ Hz, ArCH$_2$CH$_2$CO), 3.17 (t, 2 H, $J = 7.4$ Hz, CONCH$_2$CH$_2$N), 6.87–6.92 (m, 1 H, aromatic), 7.42 (d, 1 H, aromatic), 8.20 ppm (d, 1 H, aromatic); GC–MS m/z 358 (M$^+$ +2, 0.4), 357 (M$^+$ +1, 4), 356 (M$^+$, 13), 232 (52), 218 (100), 189 (95), 181 (63), 133 (41); LC–MS (ESI$^+$) m/z 357 [M+H]$^+$, 379 [M+Na]$^+$; LC–MS–MS 357: 161, 188; Anal. (C$_{37}$H$_{40}$N$_2$O$_2$·2HCl·2H$_2$O·H$_2$O) C, H, N.

4-(3-[4-Cyclohexylpiperazin-1-yl]propyl)-2H-pyrido[3,2-b][1,4]-oxazin-3(4H)-one (13): The brown semi-solid was purified by a flash column chromatography using CH$_3$Cl/CH$_2$OH (95:5) as eluent to achieve the title compound as a yellow oil (0.093 g, 26%): $^1$H NMR δ = 1.06–1.36 [m, 5 H, cyclohexyl, NCH(CH$_2$)$_2$], 1.58–1.70 [m, 358 (M$^+$ +2, 0.4), 357 (M$^+$ +1, 4), 356 (M$^+$, 13), 232 (52), 218 (100), 189 (95), 181 (63), 133 (41); LC–MS (ESI$^+$) m/z 357 [M+H]$^+$, 379 [M+Na]$^+$; LC–MS–MS 357: 161, 188; Anal. (C$_{37}$H$_{40}$N$_2$O$_2$·2HCl·2H$_2$O·H$_2$O) C, H, N.
N-[3-(4-Cyclohexylpiperazin-1-yl)propyl]-N-(2-pyridyl)acetamide (22): The brown residue was purified by column chromatography with CHCl₃/CH₃OH (98:2) as eluent to afford the title compound as a yellow semi-solid (0.179 g, 45%): ¹H NMR δ = 1.07–1.36 [m, 5 H, cyclohexyl, NCH(CH₂)₃], 1.56–1.98 [m, 9 H, cyclohexyl NCH(CH₂)₃ and CONCH₂CH₂CH₂CO], 2.28–2.43 [m, 3 H, CHN and CONCH₂CH₂CH₂CO], 2.45–2.70 [m, 10 H, 6 H, NCH, and ArCH₂CO], 2.86 (t, 2 H, J = 8.2 Hz, ArCH₂CO), 3.42–3.75 [m, 4 H, piperezine CONCH₂CH₂CO], 4.16 (t, 2 H, J = 7.1 Hz, CONCH₂CH₂CO), 6.87–6.91 (m, 1 H, aromatic), 7.41–7.43 (m, 1 H, aromatic), 8.20–8.22 ppm (m, 1 H, aromatic); GC–MS m/z 386 (M⁺ + 2, 1), 385 (M⁺ + 1, 6), 384 (M⁺, 25), 341 (65), 217 (100), 175 (58).

N-[3-(4-Cyclohexylpiperazin-1-yl)propyl]-N-(2-pyridyl)acacetamide (23): The brown residue was purified by column chromatography with CHCl₃/CH₃OH (98:2) as eluent to give the title compound as a yellow oil (0.14 g, 40%): ¹H NMR δ = 1.00–1.37 [m, 5 H, cyclohexyl, NCH(CH₂)₃], 1.58–1.95 [m, 7 H, cyclohexyl NCH(CH₂)₃ and CONCH₂CH₂CH₂CO], 1.99 (s, 3 H, CH₃CO), 2.15–2.22 (m, 11 H, 10 H, 9 H, aromatic), 2.29–2.54 [m, 10 H, 10 H, 9 H, aromatic], 3.87 [t, 2 H, J = 7.4 Hz, CONCH₂CH₂CO], 7.19–7.21 ppm (m, 2 H, aromatic), 7.71–7.77 (m, 1 H, aromatic), 8.50 ppm (m, 1 H, aromatic); ¹³C NMR (title compound as oxalate salt): δ = 21.79, 22.32, 23.44, 24.22, 66.40, 45.12, 56.24, 53.44, 66.37, 122.99, 124.74, 141.24, 149.01, 153.17, 165.84, 174.25 ppm; GC–MS m/z 345 (M⁺ + 1, 1), 344 (M⁺, 1), 219 (42), 206 (100), 181 (49), 177 (39), 135 (21); LC–MS (ESI⁺) m/z 345 (M⁺ + H)⁺, 367 ([M+Na]⁺); LC–MS–MS 345: 135, 177; Anal. (C₉₀H₇₁O₂N₂·2CH₂O₂·H₂O) C, H, N.

N-[3-(4-Cyclohexylpiperazin-1-yl)propyl]-N-(4-methoxy-2-pyridyl)acetamide (24): The yellow residue was purified by column chromatography with CHCl₃/CH₃OH (95:5) as eluent to give the final compound as a light yellow semi-solid (0.212 g, 62%): ¹H NMR δ = 1.07–1.38 [m, 5 H, cyclohexyl NCH(CH₂)₃], 1.58–2.05 [m, 9 H, cyclohexyl NCH(CH₂)₃ and ArCH₂CH₂CH₂CH₂CH₂CO], 2.28–2.50 [m, 44, 3 H, CHN, J = 7.1 Hz, N(CH₂)₂CH₃CO], 2.55–2.88 (m, 10 H, 10 H, aromatic), 3.59 [t, 2 H, J = 5.6 Hz, ArCH₂CH₂CO], 3.59 [t, 2 H, J = 7.1 Hz, N(CH₂)₂CH₃CO], 2.48–2.59 ppm (m, 3 H, CH₃N), 1.94; 1H NMR δ = 1.08–1.48 [m, 5 H, cyclohexyl NCH(CH₂)₃], 1.58–1.72 [m, 1 H, cyclohexyl NCH(CH₂)₃], 1.75–1.94 [m, 4 H, cyclohexyl NCH(CH₂)₃], 1.95–2.12 [m, 2 H, NCH₂CH₂CH₂CO], 2.42–2.59 ppm (m, 3 H, CH₃N), and 7.72–7.78 ppm (m, 1 H, aromatic).
NCH2CH2CH2N), 2.62–2.98 (m, 8H, piperazine), 3.47 (t, 2H, J = 4.54 Hz, OCH2CH2), 3.62 (t, 2H, J = 7.15 Hz, NCH2(CH2)3N), 4.19 (t, 2H, J = 4.54 Hz, OCH2(CH2)3N), 6.46–6.50 (m, 1H, aromatic), 6.86–6.92 (m, 1H, aromatic), 7.68–7.72 ppm (1m, 1H, aromatic); GC–MS m/z 346 (M+2, 0.4), 345 (M+1, 3), 344 (M+, 13), 206 (100), 163 (41), 149 (59), 97 (38); Anal. (C13H16N3O·3HCl·2H2O) C, H, N.

1-[4-(4-Cyclohexylpiperazin-1-yl)butyl]-2,3,4-tetrahydro-1,8-naphthyridine (18): The yellow residue was purified by column chromatography with CH2Cl2/CH3OH (98:2) as eluent to afford the title compound as a pale yellow oil (0.198 g, 55 %): 1H NMR 1.07–1.19 [m, 2H, J = 7.1 Hz, CH2 and cyclohexyl NCH2(CH2)3N, 1.60–1.99 (m, 7H, cyclohexyl NCH2(CH2)3N, and NCH2CH2CH2N), 2.30–2.33 (m, 1H, CHN), 2.40 (t, 2H, J = 7.1 Hz, CH2,CH2N), 2.56–2.67 (m, 8H, piperazine), 3.42–3.54 (m, 4H, CH2NCH2CH2N), 3.78 (s, 3H, OCH3), 5.93 (1H, J = 2.2 Hz, aromatic), 6.12–6.14 (dd, 1H, J = 5.7 Hz and J = 2.2 Hz, aromatic), 7.96 ppm (d, 1H, J = 5.7 Hz, aromatic); GC–MS m/z 360 (M+, 1), 222 (100), 179 (50), 165 (83), 137 (48); Anal. (C23H30N3O·3C2H4O) C, H, N.

3-(4-Cyclohexylpiperazin-1-yl)-N-Ethyl-N-(2-pyridyl)butanamine (28): The yellow solid residue was purified by column chromatography with CH2Cl2/CH3OH (95:5) as eluent to give the title compound as a pale yellow semi-solid (0.248 g, 73 %); 1H NMR δ = 0.98–1.38 [m, 4H, 8H, J = 7.1 Hz, CH2 and cyclohexyl NCH2(CH2)3N], 1.45–1.64 (m, 5H, cyclohexyl NCH2(CH2)3N), 1.70–2.05 (m, 4H, NCH2CH2CH2N), 2.22–2.44 [m, 3H, CHN and NCH2CH2CH2N], 2.50–2.80 (m, 8H, piperazine), 3.35–3.56 (m, 4H, CH2NCH2CH2N), 6.38–6.50 (m, 2H, aromatic), 7.32–7.45 (m, 1H, aromatic), 8.11 ppm (d, 1H, J = 4.9 Hz, aromatic); LC–MS (ESI+) m/z 345 [M+H]+; LC–MS–MS 345: 149, 177, 223; Anal. (C17H20N3O·2H2O) C, H, N.

3-(4-Cyclohexylpiperazin-1-yl)-N-Ethyl-N-(2-pyridyl)pentanamine (29): The yellow solid residue was purified by column chromatography with CH2Cl2/CH3OH (85:15) as eluent to give the title compound as a yellow oil (0.194 g, 54 %); 1H NMR δ = 1.06–1.38 [m+4, 10H, J = 7.1 Hz, CH2NCH2N, cyclohexyl NCH2(CH2)3N, and NCH2CH2CH2N], 1.44–1.69 (m, 5H, cyclohexyl NCH2(CH2)3N), 1.70–1.98 (m, 4H, NCH2CH2CH2CH2N), 2.20–2.38 [m+4, 3H, J = 7.9 Hz, N(CH2)2CH2N and CHN), 2.43–2.78 (m, 8H, piperazine), 3.40 (t, 2H, J = 7.4 Hz, NCH2(CH2)3N), 3.49 (q, 2H, J = 7.1 Hz, CH2CH2N), 6.40–6.47 (m, 2H, aromatic), 7.35–7.41 (m, 1H, J = 6.8 Hz, aromatic), 8.11 ppm (d, 1H, J = 4.9 Hz, aromatic); GC–MS m/z 359 (M+4, 1), 358 (M+, 5), 220 (100), 181 (34), 135 (26), 125 (43); Anal. (C18H23N3O·2H2O) C, H, N.

General procedure for the synthesis of intermediate compounds 33 and 34: Amine 32 (1.0 mmol, 0.22 g) was added to a solution of pyridine-1-oxide derivative 30 or 31 (1.0 mmol) in n-butanol (4 mL) in the presence of Et3N (1.0 mmol, 0.14 mL). The reaction mixture was heated at 120 °C for 20 h. After cooling, the solvent was evaporated under reduced pressure to give a brown oil residue.

3-(4-Cyclohexylpiperazin-1-yl)-N-(2-pyridyl)-N-oxide)propanamine (33): The brown oil was purified by column chromatography with CH2Cl2/MeOH (9:1) as eluent affording the title compound as a yellow semi-solid (0.178 g, 56 %); 1H NMR δ = 1.10–1.74 (m, 7H, cyclohexyl), 1.84–2.03 (m, 5H, 3H of cyclohexyl and NHCH2CH2N), 2.16–2.29 (m, 1H, cyclohexyl CHN), 2.69 ppm (2H, aromatic), 3.03–3.11 (m, 4H, piperazine), 3.26–3.37 (m, 6H, 4 piperazine and NHCH2N), 4.69–5.64 (m, 2H, aromatic), 7.15–7.24 (m, 1H, aromatic), 8.06–8.16 (m, 1H, aromatic), 8.26 ppm (broad s, 1H, NH, D2O exchanged); LC–MS (ESI+) m/z 319 [M+H]+; LC–MS–MS 319: 135, 181, 237.

3-(4-Cyclohexylpiperazin-1-yl)-N-(2-methoxy-2-pyridyl)-N-oxide)propanamine (34): The brown oil was purified by column chromatography with CHCl3/MeOH (9:1) as eluent affording the title compound as an orange oil (0.30 g, 86 %); 1H NMR δ = 1.11–1.78 (m, 7H, cyclohexyl), 1.90–2.16 (m, 5H, 3H of cyclohexyl and NHCH2CH2N, 2.16–2.35 (m, 1H, cyclohexyl CHN), 2.79–2.98 (m, 4H, piperazine), 3.26–3.37 (m, 6H, 4 piperazine and NHCH2N), 4.69–5.64 (m, 2H, aromatic), 6.15–6.20 (m, 1H, aromatic), 7.95–8.00 (m, 1H, aromatic), 8.06 ppm (broad s, 1H, NH, D2O exchanged); LC–MS (ESI+) m/z 349 [M+H]+; LC–MS–MS 349: 137, 165, 237.

General procedure for the synthesis of Final Compounds 35 and 36: To a suspension of intermediate 33 or 34 (1.0 mmol) in CHCl3 (15 mL) cooled at 0 °C, PCl5 (3.0 mmol, 0.26 mL) was added in a dropwise manner, and the mixture was heated for 1 h at 80 °C. After cooling, water was added, and the reaction mixture was made alkaline by adding NaOH and extracted with CHCl3 (3 x 70 mL). The collected organic solutions were dried (Na2SO4) and the crude residue was purified as reported below.

The crude yellow oil was purified by column chromatography with CHCl₃/CH₃OH (9:1) as eluent to give the title compound as a yellow semi-solid (0.160 g, 48 %): ¹H NMR δ = 1.06-1.29 [m, 5 H, cyclohexyl NCH(CH₃)₂], 1.59-1.63 [m, 1 H, cyclohexyl NCH(CH₃)], 1.73-1.91 [m, 6 H, cyclohexyl NCH₃CH₂], and NHCH₂CH₂CH₃N], 2.23-2.29 [m, 1 H, cyclohexyl CH₂N], 2.42-2.65 (m, 1 H, aromatic), 6.35 (d, 1 H, J = 8.2 Hz, aromatic), 5.60-5.65 (m, 1 H, aromatic), 7.34-7.40 (m, 1 H, aromatic), 8.04-8.08 ppm (m, 1 H, aromatic); ¹³C NMR (title compound as oxalate salt): 22.75, 24.45, 26.62, 38.69, 45.78, 49.7, 54.15, 65.33, 113.02, 135.06, 135.26, 144.09, 152.09, 166.70; LC–MS–MS 333: 137, 165; Anal. (C₁₉H₃₂N₄O·3 C₂H₂O₄·1/2 H₂O) C, H, N.

3-(4-Cyclohexylpiperazin-1-yl)-N-(2-pyridyl)propanamide (36): The crude brown semi-solid was purified by column chromatography with ETOAc and petroleum ether (8:2) as eluent to give the title compound as a yellow semi-solid (0.160 g, 48 %): ¹H NMR δ = 1.06-1.29 [m, 5 H, cyclohexyl NCH(CH₃)₂], 1.59-1.63 [m, 1 H, cyclohexyl NCH(CH₃)], 1.73-1.91 [m, 6 H, cyclohexyl NCH₃CH₂], and NHCH₂CH₂CH₃N], 2.23-2.29 [m, 1 H, cyclohexyl CH₂N], 2.42-2.65 (m, 1 H, aromatic), 6.35 (d, 1 H, J = 8.2 Hz, aromatic), 5.60-5.65 (m, 1 H, aromatic), 7.34-7.40 (m, 1 H, aromatic), 8.04-8.08 ppm (m, 1 H, aromatic); ¹³C NMR (title compound as oxalate salt): 22.75, 24.45, 26.62, 38.69, 45.78, 49.7, 54.15, 65.33, 113.02, 135.06, 135.26, 144.09, 152.09, 166.70; LC–MS–MS 333: 137, 165; Anal. (C₁₉H₃₂N₄O·3 C₂H₂O₄·1/2 H₂O) C, H, N.

Cell culture: The human SK-N-SH neuroblastoma and the human MCF-7 breast adenocarcinoma were obtained from Interlab Cell Line Collection (ICLCL, Genoa, Italy). The MCF-7-α₁ receptor line was created in our laboratory. The HT-22 cell line was a gift from Dr. Alessandra Rossi, (Heinrich–Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany). MCF-7wt, MCF-7-α₁, and HT-22 cells were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, in a humidified incubator at 37 °C with a 5% CO₂ atmosphere. SK-N-SH cell line was routinely cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, and 1% non-essential amino acids in a humidified incubator at 37 °C with a 5% CO₂ atmosphere. Cell culture reagents were purchased from EuroClone (Milan, Italy). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide), G418 (geneticin), and fura-2-acetoxymethyl ester (Fura-2-AM) were obtained from Sigma–Aldrich (Milan, Italy); FuGENE HD transfection reagent was purchased from Promega (Milan, Italy); Opti-MEM was obtained from Invitrogen.

Cell viability: Determination of cell growth was performed using the MTT assay at 48 h. On day 1, 25,000 cells per well were seeded into 96-well plates in a volume of 100 μL. On day 2, the various drugs at concentrations between 0.1–100 μM were added. In all of the experiments, the various drug solvents (ETOAc, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with drugs, MTT (0.5 mg/mL) was added to each well, and after 3-h incubation at 37 °C, the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO/ETOAc (1:1) and the absorbance values at 570 and 630 nm were determined on a Victor 3 microplate reader from PerkinElmer Life Sciences.

Construction of expression vector harboring α₁ receptor complete coding sequence (CDS): Total RNA was extracted from 1×10⁶ MCF-7wt cells using a GenElute Mammalian Total RNA Miniprep kit (Sigma–Aldrich) and reverse transcribed with GeneAmp RNA PCR core kit (Applied Biosystems). The full-length coding region of the human α₁ receptor (GenBank accession number NM_005866.2) was amplified from MCF-7wt cDNA using iProof High Fidelity DNA Polymerase (Bio-Rad), 10 pmol of each primer (Table 3) and 1 mL of dNTPs (10 mM for each nucleotide) in a final volume of 50 μL of regular buffer reaction mixture. PCR was run under the following conditions: pre-incubation at 94 °C for 1 min, run for 30 cycles at 94 °C for 10 sec, 55 °C for 30 sec, and 72 °C for 1 min, extension at 72 °C for 7 min. The PCR amplification product was purified using the High Pure PCR Product Purification kit (Roche) and digested with HindIII and BamHI (Roche). The digested DNA fragment was ligated with purified HindIII- and BamHI-digested pCDNA3.1(+) vector (Invitrogen) in the sense orientation. Top 10 chemically competent Escherichia coli cells (Invitrogen) were transformed with the
Table 3. Primers used for amplification and sequencing of α1 receptor CDS.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5'→3'</th>
</tr>
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<tbody>
<tr>
<td>SGMA1FOR</td>
<td>CCGAAAGCTATGCAGGCGGTGAGCCCTGGAGG</td>
</tr>
<tr>
<td>SGMA1REV</td>
<td>CAGGAGTCCCATCGAGGCGGTGAGCCCTGGAGG</td>
</tr>
<tr>
<td>pcDNAF</td>
<td>AATACGACTCACTATAGGG</td>
</tr>
<tr>
<td>SGMA1FOR100</td>
<td>TCCGAGATGTTGGCTGTCTT</td>
</tr>
<tr>
<td>pcDNAR</td>
<td>AGAAGGCAAGAGCGGAGG</td>
</tr>
</tbody>
</table>

construct described above and the vector amplified. The plasmid DNA was isolated using a High Pure Plasmid Isolation kit (Roche).42 The fidelity of the final human α1 receptor insert in pcDNA3.1(+) plasmid was verified by DNA sequencing using a BigDye Terminator kit (Applied Biosystems) and the primers shown in Table 3.

MCF-7 transfection with α1 receptor: To develop stable MCF-7-7α, cell lines, MCF-7wt cells were plated at a density of 3×10^5 cells in 10 mL growth medium in 100 mm Petri dishes, and incubated at 37°C overnight. Cells were transfected with 17 μg of pcDNA3.1(+) vector containing the target α1 DNA sequence as per standard protocol, using FuGENE HD transfection reagent in Opti-MEM medium without serum. Vector-expressing cells were selected using gentamicin (G418). After transfection, cells were placed in normal DMEM growth medium. After 1 day, cells were detached with trypsin/EDTA and replated into DMEM growth medium containing gentamicin (800 μg/mL).43 and cultured for 25 days. Surviving cell clones were picked out and propagated separately in 60 mm Petri dishes in the same medium with 800 μg/mL gentamicin. To suppress reversion of the phenotype, all subsequent cell culture was carried out in DMEM growth medium as described above, supplemented with 800 μg/mL gentamicin.44

Fluorescence measurements for intracellular Ca^{2+} response detection: Cells were seeded onto glass cover-slips at a density of 20,000 cm^2 and used for fluorescence measurements after 5 days. Intracellular [Ca^{2+}] was estimated using the dual-wavelength ratiometric probe Fura-2; protocols have been described elsewhere (Galiano et al., 2004).45 A HEPES buffer was used containing 120 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2, 1.6 mM MgCl_2, 11 mM glucose, and 25 mM HEPES (pH 7.4). Agonists or other drugs were directly pipetted into the chamber without perfusion. The experiment was run at 22°C. Each trace shown is the mean of values from at least eight representatives and at least 50 total cells from four experiments, performed with different cell batches; only one in four S.E.M. values was plotted. In Figure 3, "(R−R0)/R0 in 60 s" is the difference between the mean value of 12 and 10 fluorescence ratio values, measured every 5 s before and after addition of the drug under investigation. Where indicated, data were statistically analyzed with Student’s t-test for unpaired values.46

Supporting Information

Elemental analyses of the novel end products; formulas of hydrochloride and oxalate salts, crystallization solvents, melting points and ClogD values, antiproliferative effects in MCF-7wt cells of compound 4 alone and in co-administration with 18, description of the preparation and spectroscopy data for the intermediate compounds 6–10, 20, and 21.

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Keywords: 2-aminopyridines · calcium · N-cyclohexylpiperazines · α receptors

[40] Prism Software, version 3.0 for Windows, GraphPad Software Inc., San Diego, CA (USA), 1998.

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