Design, Synthesis and Biological evaluation of a bivalent $\mu$ opiate and adenosine A1 receptors antagonist

Smitha C. Mathew, Nandita Ghosh, Youlet By, Aurélie Berthault, Marie-Alice Virolleaud, Louis Carrega, Gaëlle Chouraqui, Laurent Commeiras, Jocelyne Condo, Mireille Attolini, et al.

To cite this version:


HAL Id: hal-00679622
https://hal.science/hal-00679622
Submitted on 16 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Design, Synthesis and Biological evaluation of a bivalent \(\mu\) opiate and adenosine A1 receptors antagonist

Smitha C. Mathew, a Nandita Ghosh, a Youlet By, b Aurélie Berthault, a Marie-Alice Virolleaud, a Louis Carrega, b Gaëlle Chouraqui, a Laurent Commeiras, a Jocelyne Condo, b Mireille Attolini, a Anouk Gaudel-Siri, a Jean Ruf, b Jean-Luc Parrain, a Jean Rodriguez, a Régis Guieu. a,c

a Aix-Marseille Université, Institut des Sciences Moléculaires de Marseille, iSm2 – UMR CNRS 6263, Centre Saint Jérôme, Service 531, 13397 Marseille Cedex 20, France
b Université de la Méditerranée, UMR MD2, Faculté de Médecine, boulevard Jean Moulin, 13005 Marseille, France
c Assistance Publique des Hôpitaux de Marseille, boulevard Jean Moulin, 13005 Marseille, France

Abstract— We designed and synthesized a new hetero-bivalent ligand having antagonist properties on both A1 adenosine and \(\mu\) opiate receptors with a \(K_i\) of 0.8±0.05 and 0.7±0.03 \(\mu\)M, respectively. This bipolar compound increases cAMP production both in cells over expressing the \(\mu\) receptor and in those over expressing the A1 adenosine receptor and reverses the antalgic effects of \(\mu\) and A1 adenosine receptors agonist in animals.

The cross talk between different G protein-coupled receptors (GPCRs) is the source of increasing research in the area of simultaneously targeting more than one GPCR. Most of cross talk between different GPCRs concerns cAMP production via the modulation of adenyllylcyclase activity. There is a great number of GPCRs that either stimulates or inhibits adenyllylcyclase activity, depending on the nature of the G protein (Gs, Gi or other). Among GPCRs, there is evidence that A1 ARs and MORs are implicated in such a cross talk and their activation decrease cAMP level in the target cells.

In peripheral nervous system, there is a cross-tolerance and cross withdrawal between A1 ARs and MORs, indicating that these receptors are localized on the same primary afferent nociceptors and that A1 ARs and MORs cooperate as a multiple receptor complex form. Our group has also demonstrated that the activation of MORs increased adenosine concentration in extra cellular spaces of the central nervous system, suggesting that most effects of opioids are due to adenosine release.

Based on the A1 ARs/MORs cross talk, the aim of this study was to design and to synthesize a potential hetero-bivalent A1 ARs/MORs ligand and to evaluate its biological effects. Hetero-bivalent ligand is a single chemical entity that is composed of two covalently linked pharmacophores with a dual mode of action, acting on two different receptor subtypes.

Modulating both A1 ARs and MORs could have therapeutic application in some diseases. As an example, the release of adenosine aggravated the hypotension during severe sepsis or septic shock, leading to subsequent tissue hypoperfusion and ischemia. Most of these effects are secondary to the activation of A1 adenosine receptors, explaining the absence of response to pressive amines in these patients. Furthermore, naloxone, a \(\mu\) receptor antagonist has been successfully used against the drop of blood pressure during septic shock or...
hypovolemic shock, suggesting that the release of endogenous opioids participates into the severity of these syndromes. Thus blocking both A<sub>1</sub> ARs and MORs may be of a great interest in these pathologies. Modulating both these receptors may also be of interest in the area of drug-withdrawal therapy.\textsuperscript{8}

We planed to associate Fentanyl [N-phenyl-N-(1-phenethyl-4-piperidinyl)propanamide] to the adenosine molecule itself (Figure 1). Indeed, Fentanyl is one of the most powerful opioid analgesics, with a potency approximately eighty times that of morphine and forty times that of oxycodone.\textsuperscript{9} Herein, we report the synthesis of a bivalent compound (10), its μ opioid (MORs) and A<sub>1</sub> adenosine (A<sub>1</sub>AR) receptors binding affinities and its biological properties.

Having in mind that the main approach to discovering AR agonists has been the modification of adenosine itself and that N<sub>6</sub>-cyclopentyladenosine (CPA) derivative displays high A<sub>1</sub>AR selectivity,\textsuperscript{10} we first turned our attention to the synthesis of compound 10 presenting a cyclopentyl substitution at the adenosine N<sub>9</sub>-position and an amide bond linker at the 5'-position.

**Figure 1.** Concept of hetero-bivalent A<sub>1</sub>ARs/MORs ligand

Our retrosynthetic approach for this potential hetero-bivalent ligand could involve an amidification step between the adenosine carboxylic acid 4 and the methyl amino fentanyl derivative 8 (Schemes 1 and 2).

Synthesis of acid 4 (Scheme 1) was now needed and started with the conversion of inosine 1 into the corresponding chloro acetal derivative 2. Preparation of chloroinosine began with the acetylation of inosine with acetic anhydride in the presence of triethylamine and DMAP to afford triacetylinosine in quantitative yield. The conversion into the chloride with thionyl chloride was directly followed by a deacetylation using a solution of ammonia in methanol to give the expected chloroinosine (90% yield over 3 steps). Selective protection of the two secondary alcohol functions was achieved with dimethoxypropane in presence of p-TSA to give 2 in 70%\textsuperscript{11} After being subjected to cyclopropylamine in the presence of calcium carbonate, compound 2 led to the corresponding secondary amine 3 in 98% yield. Thereafter the primary alcohol underwent an oxidation to the acid 4 under TEMPO-iodobenzene diacetate conditions\textsuperscript{12} in 65% yield. Attention was next turned to the fentanyl part of target molecule 8 (Scheme 2). Reductive amination of 4-aminoacetophenone with commercially available 1-phenethylpiperidine-4-one (5) afforded amine 6.

This secondary amine was next subjected to propionylation and led to the formation of the desired compound 7 in 95% yield.

**Scheme 1.** Synthesis of adenosyl carboxylic acid 4

\begin{align*}
\text{inosine 1} & \xrightarrow{a,b} \text{2} \\
\text{3} & \xrightarrow{d} \text{4}
\end{align*}

Reagents and conditions: (a) (1) Ac<sub>2</sub>O, DMAP, Et<sub>3</sub>N, ACN, rt, quant.; (2) SOCl<sub>2</sub>, DMF, DCM, 0 °C then reflux, 90%; (3) NH<sub>2</sub>/MeOH, 0 °C then 20 °C, quant. (b) dimethoxypropane, p-TSA, acetone, rt, 70%; (c) cyclopropylamine, DIEA, EtOH, 80°C, 98%; (d) BAIB, TEMPO, ACN/H<sub>2</sub>O, rt, 65%.

The latter in the presence of ammonium acetate and sodium cyanoborohydride furnished subunit 8 in 55% yield.\textsuperscript{11}

**Scheme 2.** Synthesis of methylaminofentanyl 8
Reagents and conditions: (a) 5, NaBH(OAc), AcOH, DCE, rt, 77%; (b) propionyl chloride, Et₃N, DCM, reflux, 95%; (c) NH₄OAc, NaBH₃CN, MeOH, 50 °C, 55%.

The synthesis of target 10 (Scheme 3) was completed in two steps by using a BOP-assisted coupling between acid 4 and amine 8 then a deprotection of the acetal moiety using aqueous trifluoroacetic acid affording the target molecule 10 with good overall yield. By using this approach this potential hetero-bivalent ligand was efficiently synthesized on milligram scale (93% HPLC purity grade) in 8 steps.

Scheme 3. Synthesis of adenosyl fentanyl 10

Reagents and conditions: (a) 7, BOP, Et₃N, THF, rt, 77%; (b) 80% TFA, rt, 32%.

This new hetero-bivalent ligand 10 was then submitted to biological evaluation. Adenosyl fentanyl 10 was first tested using nociceptive tests in animals. Adenosyl fentanyl 10 alone had no effect on latencies. Adenosyl fentanyl 10 also reversed significantly the increase of latencies induced by N₆ cyclopentyl adenosine (CPA), an A₁ AR agonist (Figure 2). Comparable results were obtained using intraperitoneal administration of drugs (data not shown). We also evaluated the acute toxicity of 10 in animals: LD 50 value was >50 µg/mouse (when icv administered) and > 500 mg/kg (when ip administered). We tested the properties of 10 to modulate cAMP production in cell culture. 10 Activated cAMP production in a dose dependent manner both in CHO K1 cells that over expressed MORs and in CHO Chem 3 cells that over expressed A1ARs, with a maximal stimulation of 29±7% and 17±3%, respectively.
Comparatively, naloxone, a MORs antagonist, increased cAMP production with a maximal stimulation of 50±16% while 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an A₁AR antagonist, increased cAMP production with a maximal stimulation of 45±8% (Figure 3). We also evaluated the affinities (Ki) of drugs tested, using binding assay on cell membranes (Table). 10 had substantial lower affinities for the A₁ARs as compared with A₁ARs antagonist DPCPX and/or lower affinities for the MORs as compared with the MORs antagonist naloxone or with the MORs agonist DAMGO. However, its affinity was sufficient to produce biological effects at reasonable concentrations.

Table 1: Ki values for drugs at the A1 AR and the MORs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ki A₁ AR</th>
<th>Ki A₂A</th>
<th>Ki MORs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPCPX</td>
<td>12.9±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naloxone</td>
<td>1.5±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAMGO</td>
<td>3.8±0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>800±57</td>
<td>&gt;40.000</td>
<td>728±37</td>
</tr>
</tbody>
</table>

DPCPX: 8-Cyclopentyl-1,3-dipropylxanthine (A1 AR antagonist)Naloxone: MORs antagonist; DAMGO: ([D-Ala2, N-Me Phe4, Gly-ol]enkephalin) (MORs agonist)

We have synthesized a hetero-bivalent ligand 10 that have antagonist properties on both A₁AR and MORs. Its affinity remained low for the two receptors but sufficient to have biological effects at reasonable concentration. 10 Reversed significantly the antalgic effects of DAMGO or CPA and activated cAMP production in both cell lines tested. To the best of our knowledge, adenosyl fentanyl 10 constitutes the first drug that blocks both MORs and A₁ARs. Multivalent-ligands are also called hybrid molecules, and are defined as chemical entities having different biological effects. There is evidence that adenosine via A₁ARs, is implicated in the modulation of opiates action in the central nervous system. Morphine inhibit Ca²⁺ dependent NT release; this inhibition is blocked by theophiline an adenosine receptors antagonist. A₁ARs and MORs are both implicated in septic or hypovolemic shock, withdrawal, mood regulation and pain. Thus to have drugs which modulate both A₁AR and MORs at one’s disposal, could be interesting in these area. After these encouraging preliminary results we have embarked in more systematic SAR study in design more active and selective hybrid targets. Theses results will be reported in due course.

Acknowledgments

The authors thank Agence Nationale pour la Recherche (ANR-05-BLAN-0175-01), CNRS, Université Paul Cézanne, Université de la Méditerranée, Faculté de Médecine, Assistance Publique des Hôpitaux de Marseille, Fondation Mérieux and Conseil Régional Provence Alpes Côte d’Azur for fundings; Johnson and Johnson laboratories for CHO K1 cells supply; Conseil Général des Bouches du Rhône are also gratefully acknowledge; Drs T. Meert and van Hijfte, and Miss Dechabanne for helpful suggestions.

References