Bioorganic & Medicinal Chemistry Letters 19 (2009) 6736–6739

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of a bivalent μ opiate and adenosine A1 receptor antagonist

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ARTICLE INFO

Article history:
Received 29 July 2009
Revised 28 September 2009
Accepted 29 September 2009
Available online 3 October 2009

Keywords:
Adenosine
Fentanyl
Bivalents antagonists
μ-Receptors
A1 receptors

ABSTRACT

The cross talk between different membrane receptors is the source of increasing research. We designed and synthesized a new hetero-bivalent ligand that has antagonist properties on both A_1 adenosine and μ opiate receptors with a K_i of 0.8 ± 0.05 and 0.7 ± 0.03 μM, respectively. This hybrid molecule increases cAMP production in cells that over express the μ receptor as well as those over expressing the A_1 adenosine receptor and reverses the antalgic effects of μ and A_1 adenosine receptor agonists in animals.

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The cross talk between different G protein-coupled receptors (GPCRs) is the source of increasing research in the area of simultaneous targeting of more than one GPCR.[1] Most of the cross talk between different GPCRs concerns cAMP production via the modulation of adenylyl-cyclase activity. There is a great number of GPCRs that either stimulate or inhibit adenylyl-cyclase activity, depending on the nature of the G protein (G_s, G_i or other). Among GPCRs, there is evidence that A1 adenosine receptors (A_1ARs) and μ opioid receptors (MORs) are implicated in such cross talk and their activation leads to a decrease in cAMP levels in the target cells.

In peripheral nervous system, there is a cross-tolerance and cross withdrawal between A_1ARs and MORs, indicating that these receptors are localised on the same primary afferent nociceptors and that A_1ARs and MORs cooperate as a multiple receptor complex.[2] Our group has also demonstrated that the activation of MORs increases adenosine concentration in the extra cellular spaces of the central nervous system, suggesting that most effects of opioids are due to adenosine release.[3]

Based on the A_1AR/MOR cross talk, the aim of this study was to synthesize a potential hetero-bivalent A_1AR/MOR ligand and to evaluate its biological effects. A 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.[1a,f,4]

Modulating both A_1ARs and MORs could have therapeutic application in some diseases. For example, the release of adenosine aggravates the hypotension during severe sepsis or septic shock, leading to subsequent tissue hypoperfusion and ischaemia. Most of these effects are secondary to the activation of A_1 adenosine receptors, explaining the absence of response to pressor amines in these patients.[5] Furthermore, naloxone, a μ receptor antagonist has been successfully used against the drop of blood pressure during septic shock[6] or hypovolemic shock,[7] suggesting that the release of endogenous opioids participates into the severity of these syndromes. Thus, blocking both A_1ARs 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.[8]

Fentanyl [*N*-phenyl-*N*-(1-phenethyl-4-piperidinyl)propanamide] is one of the most powerful opioid analgesics, with a potency approximately eighty times that of morphine and forty times that of oxycodone.[9] As such, we planned to associate fentanyl with adenosine to generate an appropriate hetero-bivalent ligand (Fig. 1). Herein, we report the synthesis of such a compound **10**, its MOR and A1AR binding affinities and biological properties.

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doi:10.1016/j.bmcl.2009.09.112

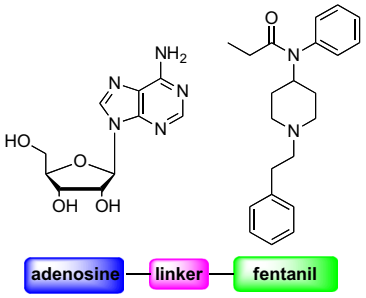


Figure 1. Concept of hetero-bivalent A_1AR/MOR ligand.

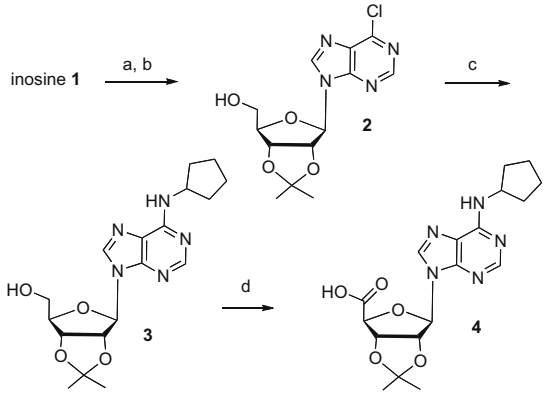
Considering that the main approach for discovering A_1AR agonists has been the modification of adenosine itself and that the N_6-cyclopentyl adenosine (CPA) derivative displays high A_1AR selectivity,[10] we first turned our attention to the synthesis of compound **10** containing an adenosine N_6-cyclopentyl group and an amide bond linker at the 5′-position.

Our approach for this potential hetero-bivalent ligand would involve amide bond formation between the adenosine and the fentanyl derivatives **4** and **8** respectively.

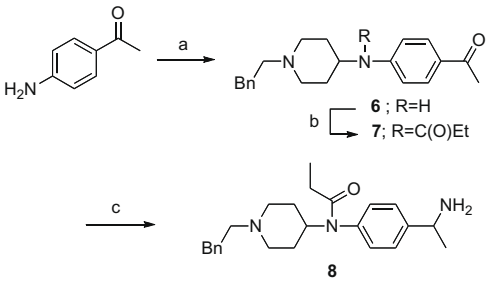
The synthesis of acid **4** (Scheme 1) commenced with the conversion of inosine (**1**) into the corresponding chloro-acetal derivative **2**. Acetylation of inosine with acetic anhydride in the presence of triethylamine and DMAP afforded the corresponding triacetylinosine in quantitative yield.

Conversion to the chloride ($SOCl_2$ in DMF) was directly followed by deacetylation using a solution of ammonia in methanol to give the expected chloroinosine (90% yield over three steps). Selective protection of the two secondary alcohols as the corresponding acetonide was achieved using dimethoxypropane in presence of *p*-TSA. Treatment of **2** with cyclopentylamine in the presence of Hunigs base provided the corresponding secondary amine **3** in 98% yield. Finally the primary alcohol was oxidised to the corresponding acid **4** under TEMPO-iodobenzene diacetate conditions[11] in 65% yield. Attention was next turned to the fentanyl subunit, as part of target molecule **8** (Scheme 2). Reductive amination of 4-aminoacetophenone with commercially available 1-phenethylpiperidine-4-one (**5**) afforded amine **6**.[12]

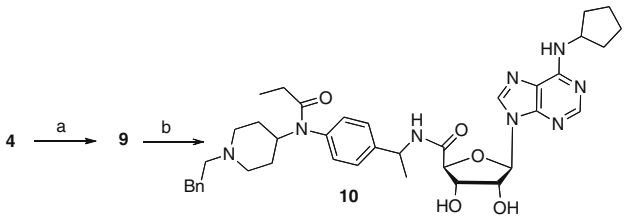
This secondary amine was next subjected to propionylation to provide **7** in 95% yield. Further reductive amination of **7** with ammonium acetate and sodium cyanoborohydride then furnished subunit **8** in 55% yield.[11]



Scheme 1. Synthesis of acid **4**. Reagents and conditions: (a) (1) Ac_2O, DMAP, Et_3N, ACN, rt, quant.; (2) $SOCl_2$, DMF, DCM, 0 °C then reflux, 90%; (3) NH_3/MeOH, 0 °C then −20 °C, quant.; (b) dimethoxypropane, *p*-TSA, acetone, rt, 70%; (c) cyclopentylamine, DIEA, EtOH, 80 °C, 98%; (d) BAIB, TEMPO, ACN/H_2O, rt, 65%.



Scheme 2. Synthesis of aminofentanyl **8**. Reagents and conditions: (a) 1-phenethylpiperidine-4-one (**5**), $NaBH(OAc)_3$, AcOH, DCE, rt, 77%; (b) propionyl chloride, Et_3N, DCM, reflux, 95%; (c) NH_4OAc, $NaBH_3CN$, MeOH, 50 °C, 55%.



Scheme 3. Synthesis of adenosyl fentanyl **10**. Reagents and conditions: (a) **7**, BOP, Et_3N, THF, rt, 77%; (b) 80% aq TFA, rt, 32%.

Completion of the targeted ligand **10** (Scheme 3) was achieved in two steps by using a BOP-assisted coupling between acid **4** and amine **8**[13] to give **9** then followed by acetal deprotection using aqueous trifluoroacetic acid. Using this approach, the potential hetero-bivalent ligand **10** was efficiently synthesized on 30 milligram scale (93% HPLC purity grade) in eight steps.[14]

This new hetero-bivalent ligand **10** was then submitted to biological evaluation. Adenosyl fentanyl **10** was first tested using nociceptive tests in mice ($n = 12$ per group, using intra cerebro ventricular route (icv)). Ligand **10** alone had no effect on latencies. However it is worthy of note that DAMGO, a well known synthetic opioid peptide with μ agonist properties, co-injected with **10** showed shorter latencies than DAMGO alone both in hot plate and tail flick tests.

Adenosyl fentanyl **10** also significantly reversed the increase in latency induced by CPA, an A1 AR agonist (Fig. 2A). Ligand **10** also reversed the increase in latencies induced by injection of both CPA and DAMGO (Fig. 2B). Comparable results were obtained using intraperitoneal (ip) administration (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. Compound **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 MOR antagonist, increased cAMP production with a maximal stimulation of 50 ± 16% while 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX), a A_1AR antagonist, increased cAMP production with a maximal stimulation of 45 ± 8% (Fig. 3). We also evaluated the affinities (K_i) of the drugs tested, using binding assay on cell membranes (Table 1). Compound **10** had substantially lower affinities for the A_1ARs as compared with A_1AR antagonist DPCPX and/or lower affinities for the MORs as compared with the MOR antagonist naloxone or with the MOR agonist DAMGO. However, its affinity was sufficient to produce biological effects at reasonable concentrations.

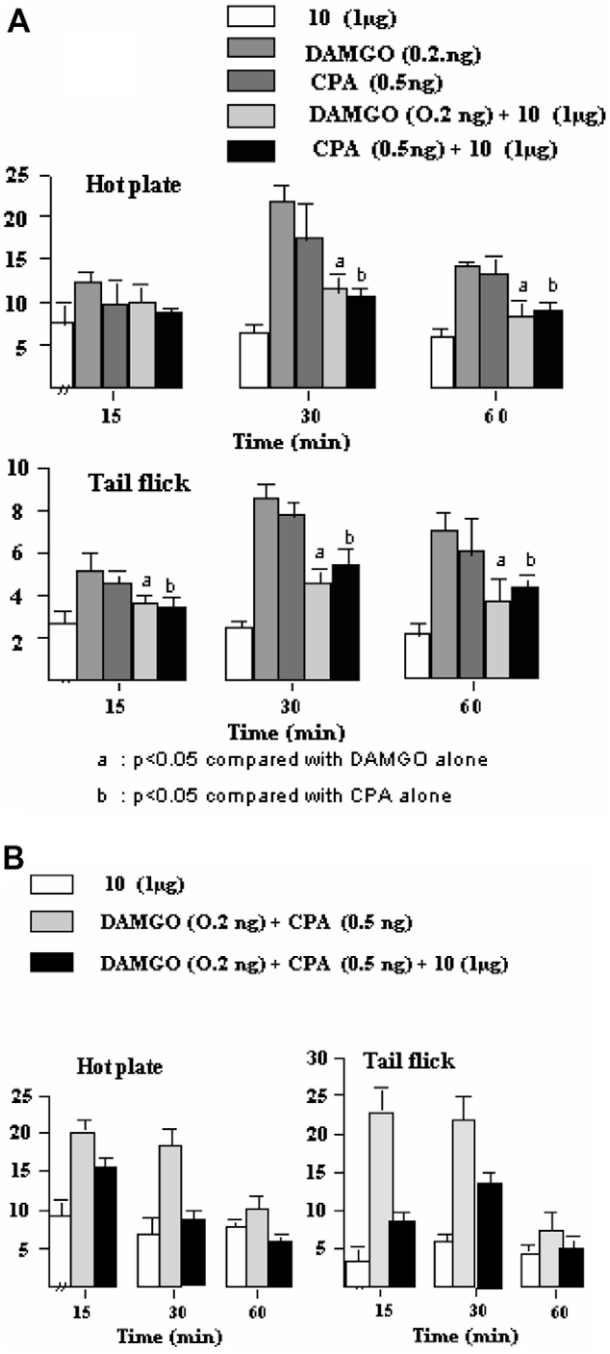


Figure 2. Results of nociceptive tests in animals.

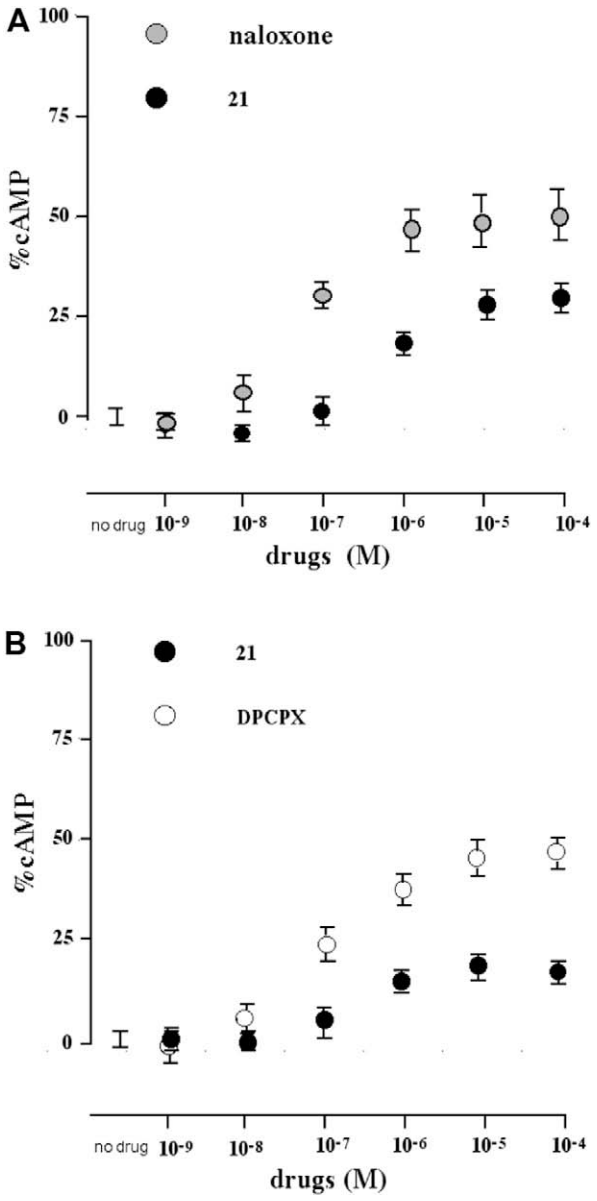


Figure 3. Comparative effects of **10**, naloxone and DPCPX on cAMP production.

Table 1
K_i values for drugs at the A_1 AR, A_{2A} ARs and the MORs DPCPX: 8-cyclopentyl-1,3-dipropylxanthine (A1 AR antagonist)

	K_i A_1ARs (nM)	K_i A_{2A}ARs (nM)	K_i MORs (nM)
DPCPX	12.9 ± 3		>100,000
Naloxone	>50,000		1.5 ± 0.2
DAMGO	>100,000		3.8 ± 0.7
10	800 ± 57	>40,000	728 ± 37

Naloxone: MOR antagonist; DAMGO: ([D-Ala2, N-Me Phe4, Gly-ol]enkephalin)(MOR agonist).

In summary, we have synthesized a hetero-bivalent ligand **10** that has antagonist properties on both A_1ARs and MORs. Its affinity remained low for the two receptors but sufficient to have biological effects at reasonable concentration. Compound **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, compound **10** constitutes the first drug that blocks both MORs and A_1ARs. Multivalent-ligands are also called hybrid molecules, and are defined as chemical entities having different biological effects.[4c] There is evidence that adenosine via A_1ARs, is implicated in the modulation of opiate action in the central nervous system.[15] Morphine[16] and adenosine[17] inhibit Ca^{2+} dependent neurotransmitter release; this inhibition is blocked by theophiline an adenosine receptors antagonist.[18] A_1ARs and MORs are both implicated in septic or hypovolemic shock,[5–7] withdrawal,[8,19] mood regulation[20] and pain[21,22] Thus drugs which modulate both A_1ARs and MORs could prove highly interesting in these therapeutic areas.

Acknowledgements

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 and Conseil Général des Bouches du Rhône are also gratefully acknowledged; Drs T. Meert and van Hijfte, and Miss De Chabanne for helpful suggestions.

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14. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05 (s, 1H), 7.98 (s, 1H), 7.33–7.07 (m, 9H), 6.93 (d, J = 12 Hz, 2H), 5.87 (d, J = 6 Hz, 1H), 5.17 (d, J = 6 Hz, 1H), 4.64–4.58 (m, 2H), 4.49 (s, 1H), 4.42 (br s, 1H), 4.29 (d, J = 6 Hz, 1H), 3.12–3.05 (m, 4H), 2.94–2.82 (m, 4H), 2.01–1.84 (m, 6H), 1.68–1.52 (m, 12H), 0.90 (t, J = 12 Hz, 3H) ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1, 170.2, 153.1, 144.6, 136.2, 130.2, 129.4, 129.0, 127.8, 90.3, 85.4, 73.5, 72.8, 58.5, 52.6, 50.4, 49.8, 49.5, 48.4, 33.5, 31.3, 30.8, 28.9, 28.0, 24.1, 21.9, 9.7; HRMS: $C_{39}H_{50}N_8O_5$ calcd: [M+H] 711.3904. Found: 711.3919.
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