Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1402–1404

Synthesis and biological characterization of [^{3}H](2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)-(4-chlorophenyl)-methanone, the first radiolabelled adenosine A_1 allosteric enhancer

Pier Giovanni Baraldi,[a,*] Maria Giovanna Pavani,[a] Edward Leung,[b] Allan R. Moorman,[b] Katia Varani,[c] Fabrizio Vincenzi,[c] Pier Andrea Borea[c] and Romeo Romagnoli[a]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy*
[b]*King Pharmaceuticals Research and Development Inc., Cary, NC 27513, USA*
[c]*Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara, 44100 Ferrara, Italy*

Received 12 October 2005; revised 10 November 2005; accepted 10 November 2005
Available online 29 November 2005

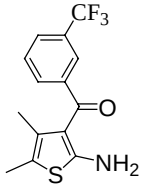
Abstract—Among the adenosine A_1 allosteric enhancers reported so far, compound (2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)-(4-chlorophenyl)-methanone **1** (named T-62) has shown biological properties similar to those of PD 81,723, the reference A_1 allosteric enhancer and it has been more fully pharmacologically investigated. The preparation of the radiolabelled form of compound **1** and its characterization by saturation binding experiments are reported. These studies allowed us to demonstrate for the first time the existence of a specific, allosteric site on the A_1 receptor.

Adenosine and adenosine A_1 agonists reduce hypersensitivity following inflammation and peripheral nerve injury in models of chronic pain.[1] However, the use of such direct agonists is complicated by a variety of adverse effects, such as angina pain, atrioventricular block, headache, nausea and retching. One approach to avoid adverse effects from direct agonists is to enhance the effect of the endogenous ligand, adenosine, by administering a positive allosteric modulator of its receptor.[2]

One class of molecules reported to act as allosteric enhancers at the A_1 adenosine receptor are the 2-amino-3-benzoylthiophene derivatives, of which PD 81,723 is the prototypical example.[3]

The closely related compound T-62 (**1**) has shown biological properties similar to those of PD 81,723.[4–7] Baraldi has described the preparation of compound T-62,[8] while Leung has described its use for treating neuropathic pain in humans.[9] T-62 is currently being investigated as a possible treatment for this disorder.[10] The promising properties of this compound prompted us to design and synthesize the radiolabelled form of T-62 (**2**).[11]

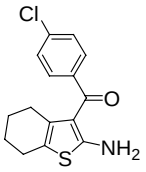
Although several approaches appeared viable, the strategy that ultimately proved successful involved the introduction of the radioisotope into the phenyl ring (Scheme 1). Recognizing that the preparation of the 4-chloro-3,5-diiodobenzoic ester **3** was known,[12] we adopted the synthetic scheme we have previously used to prepare similar 2-amino-3-aroylthiophene compounds[13] to



PD 81, 723

Keywords: Allosteric enhancer; Adenosine A_1 receptor; Radiolabelled compound.
* Corresponding author. Tel.: +39 0532 291293; fax: +39 0532 291296; e-mail: baraldi@unife.it

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.037



T-62 (1)

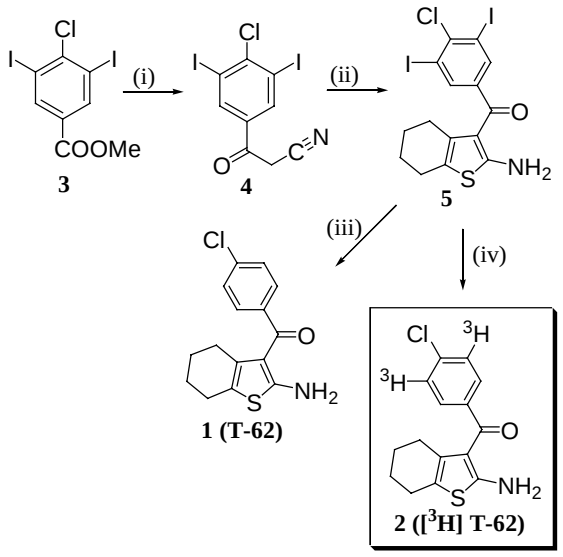
obtain derivative **5**. Condensation of the ester **3** with the anion of acetonitrile afforded the requisite β-ketonitrile (**4**). The formation of the 2-amino-3-benzoylthiophene **5** was obtained by the base-catalyzed condensation of cyclohexanone and β-ketonitrile **4**.[14] In turn, compound **5** was dehalogenated to compound **1** by catalytic hydrogenolysis and similar to compound **2** by hydrogenation in the presence of tritium gas.

The deiodination was therefore the crucial step of the synthesis. It was performed following a procedure reported in the literature for tyrosine analogues.[15] Deiodination was obtained by catalytic hydrogenolysis of derivative **5** at 50 psi in methanol/dioxane, with 10% Pd/C as catalyst to give compound **1** in good yield.[16]

Tritiation of **5** was achieved under the same conditions, affording [^{3}H]T-62 with an estimated specific activity of 47 Ci/mmol.[17]

Compound T-62 was tested in saturation binding experiments and in the functional cAMP assay[6] and it was shown to have good activity as an allosteric enhancer.

T62 caused a decrease of cAMP content of 55% at a concentration of 10 μM in CHO cells expressing the human A_1 receptor.



Scheme 1. Reagents and conditions: (i) NaH, MeCN; (ii) cyclohexanone, S, morpholine, EtOH; (iii) TEA, 10% Pd/C, H_2, 50 psi, overnight, MeOH/dioxane 2:1; (iv) TEA, 10% Pd/C, tritium, 50 psi, overnight, MeOH/dioxane 2:1.

T-62 was able to increase in a concentration-dependent way the agonist-specific binding of [^{3}H]CCPA to adenosine A_1 receptors in crude membrane preparations from CHO-A_1 cells, rat cortex and human brain, determining a 51%, 15% and 22% increase of binding in hCHO-A_1, rat cortex and human brain membranes, respectively.

T-62 increased the B_{MAX} of the agonist [^{3}H]CCPA to recombinant human A_1 adenosine receptors determining a 65%, 15% and 23% B_{MAX} increase of [^{3}H]CCPA binding in hCHO-A_1, rat cortex and human brain membranes, respectively.

T-62 did not increase the binding of the agonists [^{3}H]CGS 21680 and [^{125}I]AB-MECA on hCHO-A_{2A} and hCHO-A_3 membranes, thus behaving as a selective allosteric modulator for A_1 adenosine receptors.

The binding properties of the radiolabelled T-62 were investigated by saturation assays performed on both hCHO-A_1 cells and membranes.[18] The analysis of the experimental data[19] revealed an affinity value in the micromolar range ($K_D = 4.6 \pm 1.1$ μM) and a high receptor density ($B_{MAX} = 76.5 \pm 8.2$ pmol/mg of protein).

The biological investigation of [^{3}H]T-62 suggested the presence of an allosteric binding site on adenosine A_1 receptor distinct from the active binding site for agonists and antagonists.

The development of a new radiolabelled adenosine A_1 allosteric enhancer could provide more information about the allosteric binding site on adenosine A_1 receptor.

Acknowledgment

We thank King Pharmaceuticals Research and Development for proposal and financial support.

References and notes

1. Lee, Y. W.; Yaksh, T. L. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1642.
2. Christopoulos, A. *Nat. Rev. Drug Discov.* **2002**, *1*, 198.
3. Bruns, R. F.; Fergus, J. H. *Mol. Pharmacol.* **1990**, *38*, 939.
4. Bruns, R. F.; Fergus, J. H.; Coughenour, L. L.; Courtland, G. G.; Pugsley, T. A.; Dodd, J. H.; Tinney, F. J. *Mol. Pharmacol.* **1990**, *38*, 950.
5. Van der Klein, P. A. M.; Kouronakis, A. P.; IJzerman, A. P. *J. Med. Chem.* **1999**, *42*, 3629.
6. Baraldi, P. G.; Zaid, A. Z.; Lampronti, I.; Fruttarolo, F. F.; Pavani, M. G.; Tabrizi, M. A.; Shryock, J. C. S.; Leung, E.; Romagnoli, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1953.
7. Childers, S. R.; Li, X.; Xiao, R.; Eisenach, J. C. *J. Neurochem.* **2005**, *93*, 715.
8. Baraldi, P. G. US Patent 5,939,432, 1997.
9. Leung, E. US Patent 6,248,774, 2002.
10. Pan, H.-L.; Xu, Z.; Leung, E.; Eisenach, J. C. *Anesthesiology* **2001**, *95*, 416.

11. Pavani, M. G. Doctoral thesis at University of Ferrara, Italy, 2004, pp 58–65.
12. Borrows, E. T.; Clayton, J. C.; Hems, B. A. *J. Chem. Soc.* **1949**, *42*, 185.
13. Baraldi, P. G.; Romagnoli, R.; Pavani, M. G.; Nunez, M. C.; Tabrizi, M. A.; Shryock, J. C.; Leung, E.; Moorman, A. R.; Uluoglu, C.; Iannotta, V.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **2003**, *46*, 794.
14. Gewald, K.; Schinke, E.; Boettcher, H. *Chem. Ber* **1966**, *99*, 94.
15. Verschueren, K.; Toth, G.; Tourwé, D.; Lebl, M.; Van Binst, G.; Hruby, V. *Synthesis* **1992**, 458.
16. Procedure for deiodination reaction: A mixture of **6** (0.313 mmol, 170 mg), TEA (4 equiv, 174 μl) and 10% Pd/C (200 mg) in MeOH/1,4 dioxane (2:1 v/v, 15 ml) was reduced by Parr shaker on a high-pressure autoclave (50 psi, overnight) (TLC: ethyl acetate/petroleum ether 2:8). After filtration over Celite, the solvent was evaporated. The residue was dissolved in AcOEt , washed with water, brine and finally dried over Na_2SO_4. The evaporation of the solvent gave the crude product that was purified by column chromatography. Recrystallization from petroleum ether gave compound **1**. (Yield = 80%).
17. The synthesis of [^{3}H]T-62 was performed at Amersham International (Buckinghamshire, UK) from tritium gas through a method developed by Nycomed Amersham plc. The product was purified by high-performance liquid chromatography using a hexane/ethyl acetate gradient. The mass spectrum was consistent with the proposed structure **2** and the non-labelled reference **1**.
18. In 'hot' saturation binding experiments, a given receptor population is incubated with increasing amounts of radioligand [^{3}H]T-62 and non-specific binding is estimated at each concentration of radioligand. Non-specific binding was defined as binding in the presence of 20 μM T-62. Different incubation times (90, 120 and 180 min) and temperatures (4, 25 and 37 °C) were studied and applied to two substrates such as hCHO-A_1 cells (500.000 cells/sample) and hCHO-A_1 membranes (100 μg of protein/sample) to investigate the better experimental conditions. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass-fibre filters using a Micro-Mate 196 cell harvester (Packard Instrument Company). To reduce non-specific binding of [^{3}H]T-62 to glass fibre filter, an antiadsorbent reagent, such as polyethyleneimine at a concentration of 0.1%, was used. The filter bound radioactivity was counted on Top Count Microplate scintillation Counter (efficiency 57%) with Micro-Scint 20. In 'cold' saturation binding experiments to hCHOA_1 membranes, [^{3}H]T-62 at the concentration 10 nM was incubated for 90 min at 25 °C with different concentrations of unlabelled ligand T-62 in the range 1 nM–50 μM. Bound to free radioactivity was separated by centrifugation, a method adopted when the affinity of radioligand (K_D) to its receptor binding site is more than 10^{-8}M. See: Yamamura, H. I.; Enna, S. J.; Kuhar, H. J. In *Neurotrasmitter Receptor Binding*; Raven Press: New York, 1985; pp 61–89. In particular, the samples were centrifuged at 0 °C for 3 min at 12000*g* in a Beckman microcentrifuge and the tip of microtube containing the particulate pellet was cut off, transferred to vials containing 5 ml of Aquassure and counted by scintillation spectrometry.
19. Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.