Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1953–1957

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Effects of a New Series of 2-Amino-3-benzoylthiophenes as Allosteric Enhancers of A_1-Adenosine Receptor

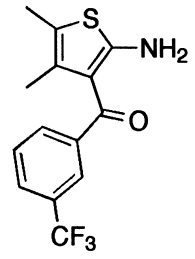
Pier Giovanni Baraldi,[a,*] Abdel Naser Zaid,[a] Ilaria Lampronti,[a] Francesca Fruttarolo,[a] Maria Giovanna Pavani,[a] Mojgan Aghazadhe Tabrizi,[a] John C. Shryock,[b] Edward Leung[c] and Romeo Romagnoli[a]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Ferrara, via Fossato di Mortara, 17/19, I-44100 Ferrara, Italy*
[b]*Department of Medicine, University of Florida, Gainseville, Florida, USA*
[c]*Medco Research, Inc., Research Triangle Park, North Carolina, USA*

Received 18 April 2000; revised 7 June 2000; accepted 26 June 2000

Abstract—New derivatives of PD 81,723, an allosteric enhancer of agonist binding to the A_1-adenosine receptor, have been synthesized and evaluated in an intact cell assay. Compounds **3a**, **3o** and **3p** appeared to be more potent than PD 81,723 and at a concentration of 0.1 μM caused significant reductions of cAMP content of CHO cells expressing the human A_1-adenosine receptor. Compounds **4e** and **4o** appeared to be allosteric enhancers at a low concentration and antagonists at a higher concentration, whereas compounds **3c**, **3g**, **3s** and **4l** appeared to be weak antagonists that are also allosteric enhancers at the higher concentration of 10 μM.

Adenosine is a ubiquitous autocoid with multiple effects on the human body. Adenosine can bind to four different P1-purinoreceptors (A_1, A_{2A}, A_{2B}, A_3) which belong to the broad group of receptors coupled to G-proteins and which modulate the activity of adenylate cyclase.[1] A_1-Adenosine receptors are found in many tissues including the heart, kidney, brain and spinal cord, adipose and thyroid tissues, lung, and immune system cells. In 1990, Bruns and co-workers[2,3] reported that 2-amino-3-benzoylthiophene derivatives both enhanced the binding of agonists to the A_1-adenosine receptor and (usually at higher concentrations) acted as competitive antagonists at these receptors. Among the synthesized compounds, PD 81,723 ((2-amino-4,5-dimethyl-3-thienyl)-[3-(trifluoromethyl)phenyl]methanone) was identified as the compound with the best ratio of enhancement to antagonistic action at the A_1-adenosine receptor.[3] To study the role of various substitutions on the phenyl ring and the importance of the 4,5-dimethyl group on the thienyl ring, Baraldi[4] and Ijzerman[5] have described the synthesis and biological evaluation of mostly novel PD 81,723 analogues. The purpose of our investigation was to synthesize and evaluate a new series of derivatives of PD 81,723 and to establish the structural requirements and the structure–activity relationship for enhancement of the action of an agonist at the human A_1-adenosine receptor.



PD 81, 723

Chemical Methods

The formation of 2-amino-3-benzoylthiophene from the base-catalyzed condensation of carbonyl compounds and nitriles, known as the 'Gewald reaction',[6] was reported 35 years ago. Nevertheless, it was not until 1990 that synthesis of 2-amino-3-benzoylthiophenes by this method received renewed attention. The same method has been used for the synthesis of our new 2-amino-3-benzoylthiophene derivatives **3a**–**x** and **4a**–**v** wherein the appropriate carbonyl compounds were reacted with benzoylacetonitrile derivatives **2a**–**h** and sulfur in ethanol in the presence of morpholine (Scheme 1).

*Corresponding author. Tel.: +39-0532-291293; fax: +39-0532-291296; e-mail: pgb@dns.unife.it

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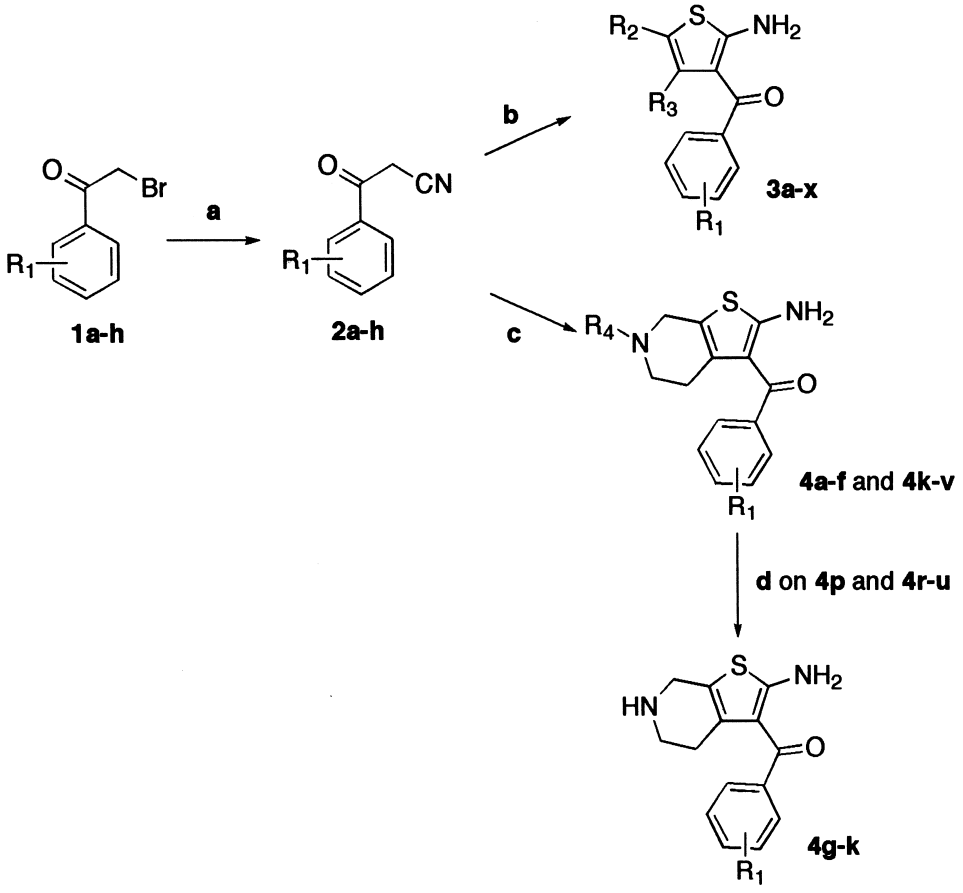
PII: S0960-894X(00)00379-6



Scheme 1. (a) KCN, EtOH, H_2O, rt, 2 h; (b) $R_2COCH_2R_3$,S_8, morpholine, EtOH, 70 °C, 1 h, rt, 18 h; (c) R_4–N⟨⟩=O, S_8, morpholine, EtOH, 70 °C, 1 h, rt, 18 h; (d) HBr/CH_3CO_2H, rt 6 h.

The derivative **4g–k** were obtained starting from **4p** and **4r–u**, respectively, removing the Cbz (Z) protective group by the treatment with HBr in acetic acid. The compounds **3c–e, 3m**, **3o–q**, **4d** have been reported by Bruns[3] and Ijzerman.[5] The benzoyl acetonitrile derivatives, when not commercially available, were synthesized by treatment of the corresponding phenacylbromides **1a–h** with potassium cyanide in high yields. The structures of the synthesized compounds and the yields of the syntheses are presented in Table 1. The range of yields was 35–67%.

Biological Methods

Chinese hamster ovary cells expressing human recombinant A_1-adenosine receptors (CHO: huA1 cells) at a density of approximately 8000 fmol/mg protein were prepared as previously described[7] and aliquots of these cells at low passage numbers were frozen and stored in liquid nitrogen. Cells were removed from liquid nitrogen storage when needed, and grown in Ham's F-12 culture medium with 10% fetal bovine serum and 0.5 mg/ml of antibiotic G-418.[8] Aliquots of cells were placed into 12-well culture plates with culture medium, serum, and antibiotic for 48–72 hours, by which time the cells had grown to a confluent monolayer. To begin an experiment, growth medium was removed from the culture plates and cells were washed once with Hanks' buffered saline solution. The wash solution was then removed and replaced with fresh Hanks' solution containing forskolin (1 μM), rolipram (20 μM), CPA (0.05–1 nM), adenosine deaminase (2 U/mL), and the allosteric enhancer to be tested. After 6 min of incubation at 36 °C in the presence of drugs, the incubation solution was removed and hydrochloric acid (final concentration, 50 mM) was added to cells to terminate drug action. The content of cAMP in acidified extracts of cells was determined by radioimmunoassay as previously described.[7] Allosteric enhancement was measured as the action of a test compound at different concentrations (0.1, 1 and 10 μM) to reduce the cAMP content of CHO: huA1 cells in the presence of 0.05–0.1 nM CPA. CPA (0.05–0.1 nM) alone causes a slight reduction of cAMP content of cells by activation of A_1-adenosine receptors. Allosteric enhancement of the action of CPA causes a further reduction of the cAMP content of CHO: huA1 cells. Because the spontaneous activity of adenosine receptors in CHO: huA1 cells causes an inhibition of adenylyl cyclase activity even in the absence of an agonist,[8] antagonists of adenosine receptors increase cAMP content of cells. Therefore, compounds that increased cAMP content of cells in this study were putatively identified as A_1-adenosine receptor antagonists. CHO: huA1 cells gradually lost responsiveness to allosteric enhancers during continuous culture. Three different batches of frozen cells were used for experiments.

Table 1. Yields for the synthesized compounds **3a–x** and **4a–v**

Compound	R_1	R_2	R_3	R_4	Yield[a](%)
3a	H	H	H		35
3b	4-Cl	H	H		45
3c[3,5]	H	CH_3	CH_3		56
3d[3,5]	3-CF_3	CH_3	CH_3		41
3e[5]	4-Cl	CH_3	CH_3		51
3f	H	-$(CH_2)_3$-			40
3g	H	-CH_2CH_2S-			35
3h	4-F	-$(CH_2)_3$-			45
3i	4-Cl	-$(CH_2)_3$-			45
3j	4-Br	-$(CH_2)_3$-			41
3k	4-I	-$(CH_2)_3$-			36
3l	4-OMe	-$(CH_2)_3$-			30
3m[5]	H	-$(CH_2)_4$-			54
3n	4-F	-$(CH_2)_4$-			46
3o[3,5]	4-Cl	-$(CH_2)_4$-			47
3p[5]	4-Br	-$(CH_2)_4$-			50
3q[3]	4-OMe	-$(CH_2)_4$-			45
3r	4-C_6H_5	-$(CH_2)_4$-			25
3s	H	-$(CH_2)_5$-			30
3t	4-F	-$(CH_2)_5$-			10
3u	4-Cl	-$(CH_2)_5$-			25
3v	4-OMe	-$(CH_2)_5$-			10
3x	4-Cl	-$(CH_2)_6$-			13
4a	3-CF_3			$C_6H_5CH_2$	51
4b	3-CF_3			$C_6H_5CH_2CH_2$	62
4c	3-CF_3			$C_6H_5CH_2CH_2CH_2$	48
4d[3,5]	4-Cl			$C_6H_5CH_2$	51
4e	4-Cl			$C_6H_5CH_2CH_2$	53
4f	4-Cl			$C_6H_5CH_2CH_2CH_2$	48
4g	H			H	58
4h	4-Cl			H	51
4i	4-Br			H	52
4j	4-I			H	38
4k	4-C_6H_5			H	62
4l	H			4-$NO_2C_6H_5CH_2CH_2$	52
4m	4-Cl			4-$NO_2C_6H_5CH_2CH_2$	59
4n	H			CH_2CO_2Et	38
4o	4-Cl			CH_2CO_2Et	63
4p	H			$CO_2CH_2C_6H_5$	66
4q	4-F			$CO_2CH_2C_6H_5$	52
4r	4-Cl			$CO_2CH_2C_6H_5$	46
4s	4-Br			$CO_2CH_2C_6H_5$	44
4t	4-I			$CO_2CH_2C_6H_5$	42
4u	4-Ph			$CO_2CH_2C_6H_5$	56
4v	H			4-$CH_3C_6H_4SO_2$	65

[a]Yield of synthesized compounds after purification by column chromatography.

Because the magnitude of the effects of allosteric enhancers on cells changed with time and batch of cells, the actions of each test compound to enhance the effect of CPA (and thus reduce cAMP content of CHO cells) were normalized to the response of cells to 10 μM PD 81,723.

Results and Discussion

The effects of the newly-synthesized derivatives of PD 81,723 on CHO cells expressing the cloned human A_1-adenosine receptor are shown in Table 2. Allosteric enhancers of agonist binding to the A_1-adenosine receptor have been shown to increase agonist binding in equilibrium binding studies,[2] to slow dissociation of the agonist-receptor complex in kinetic studies,[2] and to cause leftward shifts of the agonist concentration-response relationship in studies of isolated tissues.[7,8] In the current study, we have chosen to measure the effect of the PD 81,723 analogues on cAMP content of CHO cells expressing the cloned human A_1-adenosine receptor. This assay assessed the overall functional effect and the potential for each tested compound as an enhancer or an antagonist of the A_1-adenosine receptor. Although PD 81723 inhibited basal adenylyl cyclase (AC) activity,[9] preliminary results showed that none of synthesized compounds was interesting as inhibitor of adenylate cyclase in FRTL-5 cells.

Table 2. Allosteric enhancement by **3a–x** and **4a–v**[a]

Compound	Percentage of response to 10 μM of PD81,723 to different concentrations of tested compounds		
	0.1 μM	1 μM	10 μM
3a	51	26	28
3b	7	8	25
3c[3,5]	/[b]	*[c]	19
3d (PD 81,723)[3,5]	13	40	100
3e[5]	30	52	92
3f	35	29	82
3g	*	/	68
3h	/	43	65
3i	*	/	/
3j	31	53	72
3k	*	*	/
3l	/	43	42
3m[5]	16	48	77
3n	/	43	83
3o[3,5]	57	58	86
3p[5]	50	67	67
3q[3]	/	44	71
3r	14	79	68
3s	*	29	54
3t	26	36	61
3u	25	42	72
3v	/	54	36
3x	24	39	68
4a	18	41	85
4b	/	/	*
4c	/	7	22
4d[3,5]	/	*	*
4e	58	32	*
4f	8	15	31
4g	/	9	12
4h	15	12	9
4i	/	/	/
4j	/	*	*
4k	/	/	*
4l	*	13	31
4m	*	*	*
4m	*	13	31
4n	*	*	*
4o	10	*	*
4p	*	*	*
4q	/	*	*
4r	/	*	*
4s	*	*	*
4t	/	/	/
4u	14	6	/
4v	*	*	*

[a]The results are the average of quadruplicate determinations in each assay.
[b](/) Signifies a change of less than 5% of allosteric enhancement.
[c](*) Signifies an increase of cAMP content of cells, and may indicate antagonism of the A_1 adenosine receptor.

Many of the tested compounds appeared to be enhancers (e.g. most compounds from **3a** to **4a**) whereas others appeared to be antagonists of the A_1-adenosine receptor (e.g. most compounds from **4j** to **4v**). Although none of the tested compounds had a greater efficacy than PD 81,723, compounds **3a**, **3o** and **3p** appeared to be more

potent than PD 81,723. Because the known allosteric enhancers are also A_1-adenosine receptor antagonists at some (usually high) concentration, the fact that none of the tested compounds had a greater efficacy than did 10 μM PD 81,723 could be explained by a potential antagonist effect of the compounds at high concentrations.

We have systematically modified PD 81,723 in this study. The absence of the trifluoromethyl moiety on the phenyl ring (compound **3c**[3,5]) led to a loss of activity at any concentration, while in the same compound **3c** the removal of both methyl substituents in positions 4 and 5 on the thiophene ring, to yield compound **3a**, led to an increase of activity at a lower concentration (0.1 μM). However, compound **3a** was less active than PD 81,723 at 1 and 10 μM. No increase of activity was obtained with the presence of a non-polar substituent (such as chloro) in the *para* position on the benzoyl ring (compound **3b**). In the series of 4,5-dimethyl thiophene derivatives that we have synthesized, only the already reported compound **3e**[5] possessed an activity comparable with that of PD 81,723 at each concentration tested.

The effect of substitutions at the 4- and 5-positions of the thiophene ring in the PD 81,723 and particularly on its 'cyclized' analogues, was carefully examined. The bridging of the 4- and 5-positions in compound **3c** with a methylene, to give **3f**, caused an increase of activity compared to that reported for **3c**[3,5] at 0.1 and 10 μM, and comparable with that described for PD 81,723. When the methylene in position 6 of the dihydrocyclopentadien[*b*]thiophene ring of compound **3f** was substituted with an atom of sulfur, to give **3g**, we observed a loss of activity at 0.1 and 1 μM. However, at 10 μM compound **3g** was only slightly less effective than PD 81, 723.

We have synthesized a large series of 'cyclic' PD 81,723 analogues, represented by the dihydrocyclopentadien[*b*]thiophene derivatives **3f** and **3h–l**. As was just noted, compound **3f** was only slightly less active as an enhancer than PD 81,723. The addition of a halogen (compounds **3h–k**) or a methoxy (compound **3l**) substituent at position 4 of the phenyl ring (R_1) of compound **3f** did not lead to an increase of activity. Activity increased when the size of the fused ring (R_2–R_3) was increased from five (compounds **3f** and **3h–l**) to six (compounds **3m–r**) carbons and it decreased slightly when the size of the ring was increased from six to seven (compounds **3s–u**) carbons. Further increasing the size of the fused ring from seven to eight (compounds **3v–x**) carbons did not alter activity. However, the cyclic analogues **3f–x** were less active than PD 81,723 at a concentration of 10 μM, and only the previously reported compounds **3o**[3,5] and **3p**[5] appeared to be more potent than PD 81,723 at lower concentrations (0.1 and 1 μM). Comparing the compounds having a substituent on the benzoyl ring (R_1), the tetrahydrobenzo[*b*]thiophene derivatives **3m–r** appeared to be more potent than the dihydrocyclopentadien[*b*]thiophene counterparts **3f–l**.

An atom of nitrogen was inserted into the 6 position of the tetrahydrobenzo[*b*]thiophene ring (Scheme 1, route c) to give the 4,5,6,7-tetrahydrothieno [2,3-*c*]pyridine derivatives **4a–u**. In the series of compounds **4a–c**, which bear the 3-trifluoromethyl substituent of PD 81,723 and different phenylalkyl moieties on position six, the benzyl moiety was the best substituent. Indeed, only the *N*-benzyl derivative **4a** possessed an activity comparable to PD 81,723 at the concentration of 10 μM, while compounds **4b** and **4c** were inactive. Replacement of a 3-trifluoromethyl (compounds **4a–c)** with a 4-chloro (compounds **4d–f**) substituent on the benzoyl group led to a complete loss of activity for compound **4d**[3,5] compared to the compound **4a**. In contrast, at concentrations of 0.1 and 1 μM the derivatives **4e** and **4f** were more potent than **4b** and **4c**, respectively. The presence of a polar 4-nitro group on the phenylethyl moiety of compound **4e**, to yield compound **4l**, led to an enhancing activity which was comparable to that reported for **4f** at the concentration of 10 μM. For the compound **4d**, the removal of the only benzyl group (to form compound **4h**) and both of the *N*-benzyl and 4-chloro substituents (to form compound **4g**) had little effect on enhancing activity. When the 4-chloro substituent of the benzoyl group of compound **4h** was substituted by either a bromo, iodo or phenyl substituent, to yield the compounds **4i–k**, respectively, or the *N*-positive charge was suppressed by addition of a tosyl group (compound **4v**), no improvement in enhancing activity was observed. It has been reported by Bruns[3] that a positive charge on the nitrogen reduces enhancing activity. In fact, the *N*-ethoxycarbonyl derivatives of the compounds **4g** and **4h** showed a very potent enhancing activity. Bruns' observation was confirmed by the synthesis of the ethoxycarbonyl methyl derivatives of compounds **4g** and **4h**, corresponding to compounds **4n** and **4o**, respectively, wherein a methylene spacer has been inserted between the nitrogen and the ethoxycarbonyl moiety. These compounds contain a positively-charged nitrogen and had no enhancing activity.

Modification of the ethoxycarbonyl moiety (position R_4, Scheme 1) reduced enhancing activity. Compounds **4p–u**, which possess the *N*-benzyloxycarbonyl protective group (Z) in place of the ethoxycarbonyl moeity, were completely inactive. Of the 45 compounds synthesized for this study, several were synthesized and studied by Bruns[3] and Ijzerman[5] (see Tables 1 and 2). Our results are generally consistent with the results reported by these investigators, even though different assays and different sources of adenosine receptors were used in these studies. However, it should be noted that our assay of effects of putative enhancers on cAMP content of CHO cells expressing human A_1-adenosine receptors is not a specific assay of allosteric enhancement of agonist binding. Our assay does not directly measure the interaction between receptor activation and G protein activation, and our observations may be complicated by drug actions not related to enhancement, such as cell toxicity. However, the effect of a tested compound in the intact cell cAMP assay used in this study may be a more useful predictor of the effect of the compound in vivo than is a binding assay that more specifically assesses allosteric enhancement.

In conclusion, compounds **3a**, **3o** and **3p** appeared to be nearly as efficacious and more potent than PD 81,723,

and caused significant reductions of cAMP content of CHO: huA1 cells at a concentration of 0.1 μM. Other compounds mimicked the action of the antagonist CPX, and therefore may be antagonists of the A_1-adenosine receptor. The dual actions of benzylthiophenes to antagonize and enhance the actions of A_1-adenosine receptor agonists have been noted previously.[2,3,7,8]

Acknowledgement

We wish to thank Medco Research for support of this research.

References

1. Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413.
2. Bruns, R. F.; Fergus, J. H. *Mol. Pharmacol.* **1990**, *38*, 939.
3. 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.
4. Baraldi, P. G. US Patent 5,939,432. 1997.
5. Van der Klein, P. A. M.; Kourounakis, A. P.; Ijzerman, AdP. *J. Med. Chem.* **1999**, *42*, 3629.
6. (a) Gewald, K. *Chem. Ber.* **1965**, *98,* 3571. (b) Gewald, K.; Schinke, E.; Bottcher, H. *Chem. Ber.* **1966**, *99,* 94. (c) Peet, N. P.; Sunder, S.; Barbuch, R. J.; Vinogradoff, A. P. *J. Het. Chem.* **1986**, *23,* 129.
7. Kollias-Baker, C. A.; Ruble, J.; Jacobson, M.; Harrison, J. K.; Ozeck, M. J.; Shryock, J. C.; Belardinelli, L. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 761.
8. Shryock, J. C.; Ozeck, M. J.; Belardinelli, L. *Mol. Pharmacol.* **1998**, *53*, 886.
9. Musser, B.; Mudumbi, R. V.; Liu, J.; Olson, R. D.; Vestal, R. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 446.