Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 558–561
Bioorganic & Medicinal Chemistry Letters

# Novel nucleotide triphosphates as potent $P2Y_2$ agonists with enhanced stability over UTP

Richard J. Davenport,[a,*] Paloma Diaz,[a] Frances C. A. Galvin,[a] Steve Lloyd,[a] Stephen R. Mack,[a] Ray Owens,[b] Verity Sabin[a] and Joanne Wynn[a]

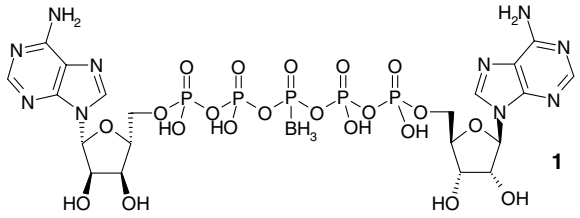
[a]*UCB-Group, Granta Park, Abington, Cambridge CB1 6GS, UK*
[b]*Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK*

Received 30 August 2006; revised 16 October 2006; accepted 16 October 2006
Available online 19 October 2006

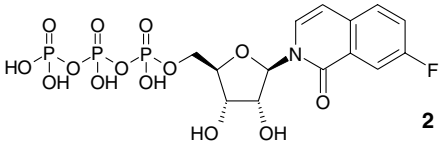
**Abstract**—The synthesis of a series of novel C-linked nucleotide triphosphates is reported. These exhibit excellent agonist potency and selectivity for the $P2Y_2$ receptor with a number of examples having $EC_{50}$ values below 10 nM. Representative compounds from the N-linked and C-linked series showed enhanced metabolic stability compared with that of the natural ligand UTP.
© 2006 Elsevier Ltd. All rights reserved.

Activation of $P2Y_2$ receptors has potential therapeutic utility in aiding mucociliary clearance and stimulating tear production.[1,2] UTP is a potent natural agonist for the $P2Y_2$ receptor, but is chemically unstable, readily metabolised and consequently has a very short duration of action. Inspire Pharmaceuticals have developed the $P2Y_2$ agonists INS365 and INS37217 for dry eye disease and cystic fibrosis, respectively.[3] Their enhanced stability compared to UTP is attributed to them being capped tetraphosphates.[4,5] It has recently been reported that the diadenosine penta(borano)phosphate **1** is a potent $P2Y_1$ agonist with greatly enhanced metabolic stability, achieved by the presence of a borano group at the enzymatic cleavage site.[6] We considered whether the use of nucleotide triphosphates bearing unnatural bases could maintain good agonist potency against the $P2Y_2$ receptor and also improve stability. In the preceding article[7] we disclosed a series of N-linked compounds, such as the 7-fluoroisocarbostyril nucleotide triphosphate **2**, which had $P2Y_2$ agonist potency comparable with that of the natural ligand UTP.

This article describes the further development of that work, investigating whether a nucleotide triphosphate incorporating a C-linked unnatural base could offer potency comparable to that of the N-linked series, and comparing the stabilities of an N- and a C-linked triphosphate to that of UTP.



**1**



**2**

Three different C-linked unnatural bases, benzothiazole, benzoxazole and benzimidazole, were selected for this work. The benzothiazole and benzoxazole examples were all synthesised from the same key intermediate, the commercially available tribenzoyl-protected cyanoribose **3**. In the case of the benzothiazoles, **3** was reacted directly with the appropriate 2-aminothiophenol[8] under basic conditions to yield the desired tribenzoyl-protected C-linked benzothiazole nucleoside **4**. If the appropriate thiophenol starting material was not available then **3** was hydrolysed to the amide, converted to the thioamide using Lawesson's Reagent[9] and then reacted with the appropriate 2-iodoaniline in the presence of palladium[10] to yield the protected C-linked nucleoside **4** (Scheme 1).

*Keywords*: $P2Y_2$; UTP; Enhanced stability.
* Corresponding author. E-mail: richard.davenport@ucb-group.com

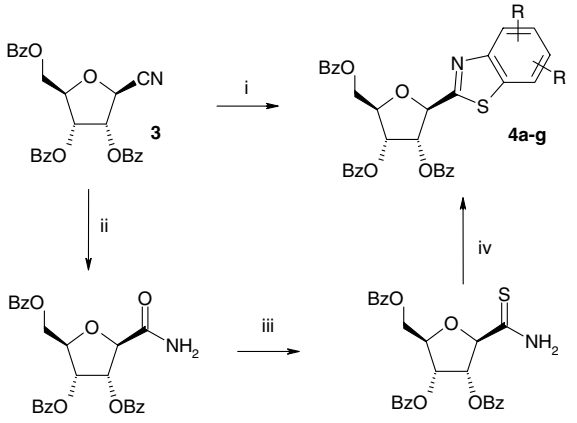
0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.038

The benzimidazole **6** was synthesised from a tribenzoyl-protected ribose carboxylic acid **5**. This was converted to the acid chloride, which was coupled with 1,2-diaminobenzene to yield an amide intermediate. Ring closure was achieved using $POCl_3$, and the free NH methylated using sodium hydride and methyl iodide to yield the desired product (Scheme 2).

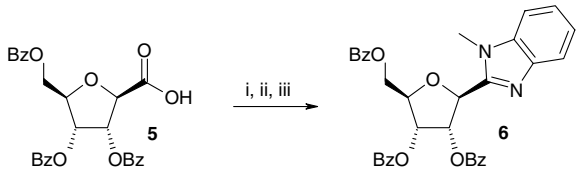
The tribenzoyl-protected C-linked benzoxazole nucleoside **7** was synthesised by reacting the key intermediate **3** with methanolic HCl[11] to yield an amidate ester, which was converted to the benzoxazole by reacting with 2-amino-4-methylphenol[12] under basic conditions (Scheme 3).

The tribenzoyl-protected compounds (**4a–g**, **6** and **7**) were treated with sodium methoxide in methanol to remove the benzoyl esters and the resulting nucleosides converted to triphosphates **8a–i** using standard conditions[13] (Scheme 4). The desired triphosphates were separated from the crude reaction mixtures by preparative HPLC and isolated as the ammonium salts. Incorporation of the triphosphate unit was confirmed by $^{31}P$ NMR spectroscopy and LC-MS.

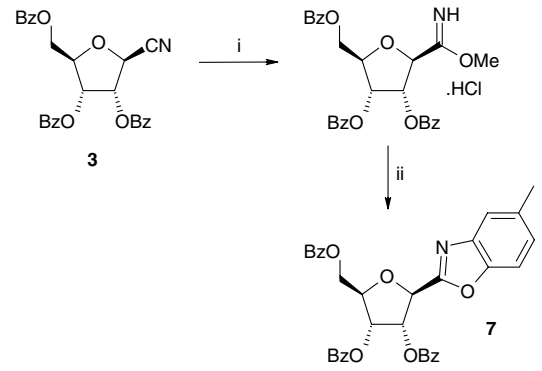
The $P2Y_2$ agonist potencies for the C-linked compounds **8a–i** are shown in Table 1. In general, the benzothiazoles were well tolerated, with several examples giving very active compounds (**8b**, **8c** and **8d**) with



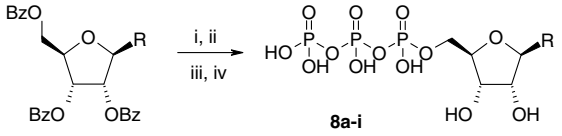

**Scheme 1.** Reagents and conditions: (i) appropriate 2-aminothiophenol, $Et_3N$, EtOH, reflux; (ii) HCl, dioxane; (iii) Lawesson's reagent, dioxane, reflux; (iv) appropriate 2-iodoaniline, $PdCl_2$, CaO, DMF 80 °C.




**Scheme 2.** Reagents and conditions: (i) $(COCl)_2$, $Et_3N$, 1,2-diaminobenzene; (ii) $POCl_3$; (iii) NaH, MeI, DMF.




**Scheme 3.** Reagents and condition: (i) HCl, MeOH; (ii) 2-amino-4-methylphenol, $Et_3N$, EtOH, reflux.




**Scheme 4.** Reagents: (i) NaOMe, MeOH; (ii) $POCl_3$, $P(OMe)_3$, proton sponge; (iii) $H_4P_2O_7{\cdot}1.5(Bu_3N)$, $Bu_3N$, DMF; (iv) $NH_4Cl$, $H_2O$.

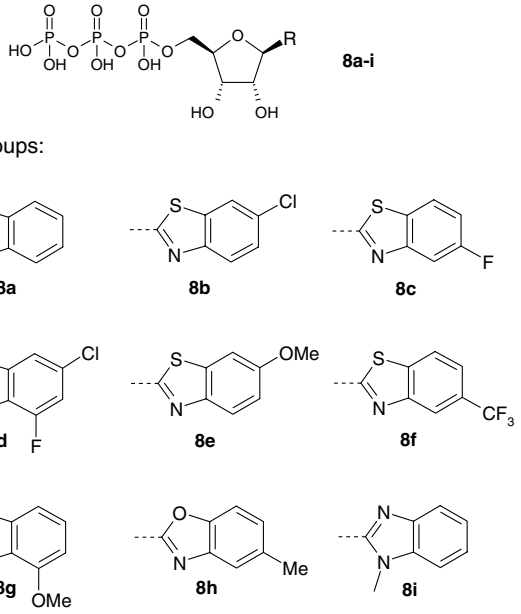
$P2Y_2$ agonist potencies similar to that of UTP. Interestingly a larger substituent in the 4-position in the case of **8g** (methoxy) dramatically reduced activity, whereas when this was much smaller, as in the case of **8d** (fluoro), the impact is far less detrimental. The benzoxazole **8h** and the benzimidazole **8i**, though tolerated, led to reduced $P2Y_2$ activity compared with those of benzthiazoles and N-linked isocarbostyrils.[7] We believe this is the first time that C-linked nucleotide triphosphates have been reported, that have levels of $P2Y_2$ potency comparable to that of the natural ligand UTP.

Some key compounds were tested against three other purinergic receptor subtypes, $P2Y_1$, $P2Y_4$, and $P2Y_6$. The results of these experiments are shown in Table 2.

All compounds tested were inactive against $P2Y_1$. While UTP had significant agonist activity at the $P2Y_4$ and $P2Y_6$ receptors our nucleotide triphosphates incorporating C-linked unnatural bases had no activity against $P2Y_6$ and, at worst, only micromolar activity against $P2Y_4$. Like our N-linked unnatural nucleotide triphosphates,[7] they therefore appear to provide a more selective template than the uracil-derived $P2Y_2$ agonists.

Having generated some potent and selective $P2Y_2$ agonists, we were interested to assess the stability of these compounds in comparison to that of the natural ligand UTP. Stability was examined using a Human Bronchial Epithelial Cell assay.[16] UTP was compared with our N- and C-linked triphosphates **2** and **8a**. The results of this

**Table 1.** Agonist potencies of compounds **8a–8f** compared with those of UTP and INS365 **2**




| Compound | $P2Y_2EC_{50}$[14] (nM) |
|---|---|
| UTP | 7 |
| **2** | 5 |
| **8a** | 22 |
| **8b** | 8 |
| **8c** | 3 |
| **8d** | 7 |
| **8e** | 42 |
| **8f** | 10 |
| **8g** | 700 |
| **8h** | 390 |
| **8i** | 200 |

**Table 2.** Agonist potencies against some other P2Y receptor subtypes

| Compound | $P2Y_1$ $EC_{50}$[15] (nM) | $P2Y_4$ $EC_{50}$[15] (nM) | $P2Y_6$ $EC_{50}$[15] (nM) |
|---|---|---|---|
| **UTP** | >2000 | 39 | 424 |
| **8a** | >20,000 | 4121 | >20,000 |
| **8d** | >20,000 | 4233 | >20,000 |
| **8f** | >20,000 | >20,000 | >20,000 |

study are shown in Figure 1. In our hands UTP was essentially all gone after 120 min. However, both the N- and C-linked unnatural UTP analogues offered enhanced stability. In the case of the C-linked compound **8a**, after 120 min there was approximately 50% unmetabolised parent remaining, with at least 20% remaining after 180 min. The result for the N-linked compound **2** was even better, with approximately 70% parent remaining after 120 min and 50% remaining at 180 min. This increased stability should lead to an increased duration of pharmacological action. One could also envisage that preparation of the dinucleotide tetraphosphate analogue of either **2** or **8a** would maintain potency and further increase stability, as was the case with INS365 compared with UTP.

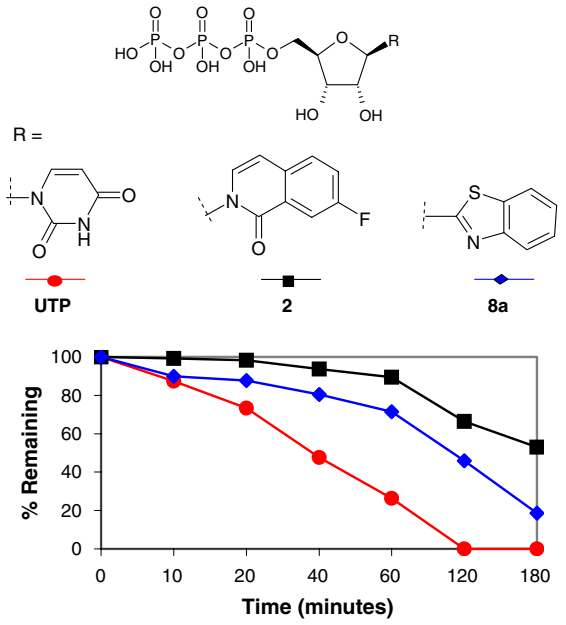



**Figure 1.** Stability comparison of N- and C-linked nucleotide triphosphates with UTP.

In conclusion, a number of novel UTP analogues have been prepared and their agonist potencies against some purinergic receptors determined. A number of these were well tolerated, having agonist potencies comparable to that of UTP. The metabolic stabilities of examples from our N- and C-linked series were compared to that of UTP, and both of our compounds were found to be significantly more stable than UTP. We believe that this enhanced stability might lead to an enhanced duration of action should these compounds be used in a therapeutic setting.

## Acknowledgments

The authors thank Slough and Cambridge NCE Project Biology groups.

## References and notes

1. Fischer, B. *Exp. Opin. Ther. Patents* **1999**, *9*, 385.
2. Yerxa, B. R.; Johnson, F. L. *Drugs Future* **1999**, *24*, 759.
3. Pendergast, W.; Yerxa, B. R.; Douglass, J. G.; Shaver, S. R.; Dougherty, R. W.; Redick, C. C.; Sims, I. F.; Rideout, J. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 157.
4. Shaver, S. R.; Rideout, J. L.; Pendergast, W.; Douglass, J. G.; Brown, E. G.; Boyer, J. L.; Patel, R. I.; Redick, C. C.; Jones, A. C.; Picher, M.; Yerxa, B. R. *Purinergic Signal.* **2005**, *1*, 183.
5. Yerxa, B. R.; Sabater, J. R.; Davis, C. W.; Stutts, M. J.; Lang-Furr, M.; Picher, M.; Jones, A. C.; Cowlen, M.; Dougherty, R.; Boyer, J.; Abraham, W. M.; Boucher, R. C. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 871.

6. Nahum, V.; Tulapurkar, M.; Levesque, S. A.; Sevigny, J.; Reiser, G.; Fischer, B. *J. Med. Chem.* **2006**, *49*, 1980.
7. Brookings, D.; Davenport, R. J.; Davis, J.; Galvin, F. C. A.; Lloyd, S.; Mack, S. R.; Owens R.; Sabin, V.; Wynn, J.; *Bioorg. Med. Chem. Lett.* **2006**, doi:10.1016/j.bmcl.2006.09.017.
8. Mylari, B. L.; Llarson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Singleton, D. H. *J. Med. Chem.* **1991**, *34*, 108.
9. Brown, T. J.; Chapman, R. F.; Cook, D. C.; Hart, T. W.; McLay, I. M. *J. Med. Chem.* **1992**, *35*, 3613.
10. Takagi, K.; Iwachido, T.; Hayama, N. *Chem. Lett.* **1987**, 839.
11. Connolly, D. J.; Lacey, P. M.; McCarthy, M.; Saunders, C. P.; Carroll, A.-M.; Goddard, R.; Guiry, P. J. *J. Org. Chem.* **2004**, *69*, 6572.
12. El Khadem, H. S.; Kawai, J. *Carbohydr. Res.* **1986**, *153*, 271.
13. Nawrot, B.; Hoffmueller, P.; Sprinzl, M. *Chem. Pap.* **1996**, *50*, 151.
14. The assay used was a calcium response assay performed in a FLIPR™ using cloned human $P2Y_2$ receptor expressed in a human astrocytoma cell line, 1321 N1.
15. Cloned Human $P2Y_1$, $P2Y_4$ and $P2Y_6$ receptors were stably expressed in 1321 N1 cells and assayed as described for $P2Y_2$. Values are means of at least four experiments.
16. Compounds were incubated at 38 °C with Human bronchial epithelial cells. Aliquots were taken at 10 min intervals, the cells lysed followed by the addition of methanol to stop and fix the reaction. Samples were then stored at 0 °C until analysis was performed. Analysis of UTP (retention time 4.9 min) was performed using a Thermohypersil BIOBASIC 150 * 4.6 mm 5 μm column, 40% A: 60% B; isocratic system; solvent A: aqueous 5 mM $KH_2PO_4$ pH 5.0; solvent B: aqueous 0.75 M $KH_2PO_4$ pH 5.0; run time 20 min; detector wavelength 254 nm. Analysis of N-linked (retention time 6.8 min) and C-linked (retention time 7.2 min) triphosphates was performed using a Develosil RPaqueous 150 * 4.6 mm 5 μm column, 100% A: 0% B to 50% A: 50% B; isocratic system; solvent A: aqueous 0.1 M TEAB, pH 7.5; solvent B: MeCN; run time 16 min; detector wavelength 254 nm. The data presented are means of two separate stability runs.