Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 977–979

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Benzimidazole-4,7-diones as Inhibitors of Protozoal (*Toxoplasma gondii*) Purine Nucleoside Phosphorylase

Frédéric Alvarez,[a] Arnaud Ghérardi,[b] Pascal Nebois,[a] Marie-Elizabeth Sarciron,[b] Anne-Françoise Pétavy[b] and Nadia Walchshofer[a,*]

[a]*Laboratoire de Chimie Organique (EA 635), Faculté de Pharmacie, Université Lyon I, 8, Avenue Rockefeller, 69373 Lyon Cedex 08, France*

[b]*Laboratoire de Parasitologie (EA 1887), Faculté de Pharmacie, Université Lyon I, 8, Avenue Rockefeller, 69373 Lyon Cedex 08, France*

Received 8 October 2001; revised 21 December 2001; accepted 22 January 2002

Abstract—Benzimidazole-4,7-diones derivatives substituted at 1- and/or 2-position have been synthetized and tested as inhibitors of purine nucleoside phosphorylase (PNP), isolated from two strains of *Toxoplasma gondii* (RH and ME 49). They were identified as inhibitors of both enzymes.

Toxoplasma gondii is a unicellular protozoan which causes severe disease in immunocompromized patients. Toxoplasmic encephalitis is a common cause of mortality in AIDS patients, either by primary infection, or by reactivation of CNS tissue cysts.[1] Only a few drugs are active against this parasite, and they lead to severe side effects.[2] Progress in treatment of toxoplasmosis with more potent or less toxic drugs is required and implies a greater knowledge of the parasite biology to determine novel chemotherapeutic targets.

All classified protozoa lack the de novo purine biosynthesis and require host-supplied preformed purines.[3] Purine salvage pathway is therefore crucial for the parasite survival. Purine nucleoside phosphorylase PNP (E.C. 2.4.2.1) is a ubiquitous key enzyme in this metabolic way.[4] It reversibly catalyses the phosphorolysis of purine nucleosides to corresponding bases and sugar 1-phosphate.

We have recently characterized the PNP of *T. gondii*.[5] It possesses properties different from those of other species and may be therefore a potential target for antiparasitic compounds.

Many inhibitors of PNP, mainly of the human erythrocyte and calf spleen enzymes, have been described.[4,6] They are closely related to purine structure, with a pyrimidinone ring fused to an imidazole, a pyrole or a triazole nucleus.

On the other hand, heterocyclic quinones, like naphto[2,3-*d*]imidazole-4,9-dione, have been described as inhibitors of hypoxanthine phosphoribosyltransferase HGPRT (E.C. 2.4.2.8), another enzyme of the purine salvage pathway.[7]

Thus, we decided to synthetize benzimidazole-4,7-dione derivatives **8a**–**j** (Table 1), in order to evaluate their ability to inhibit in vitro *T. gondii* purine nucleoside phosphorylase from two strains, a virulent strain RH and a cystic strain ME 49.

PNP inhibition activity of **8a**–**j** was compared to that of three reference compounds: 8-aminoguanosine (AG), a *Plasmodium falciparum* and human PNP inhibitor,[9] formycine A (FoA), an *Escherichia coli* PNP inhibitor,[6b] and allopurinol riboside (alloR), an inhibitor of human PNP, but not of *Plasmodium lophurae* PNP[6a] (Fig. 1).

Chemistry

The precursors, compounds **1**–**2**,[10] **3**–**4**,[8] **5**,[11] **6a**–**7a** and **6g**–**7g**,[8] are reported in the literature.

Benzimidazolediones **8a–j** were synthetized according to procedures modified with regard to those published.

*Corresponding author. Fax +33-4-7877-7158; e-mail: nadia.walchshofer@univ-lyon1.fr

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00064-1

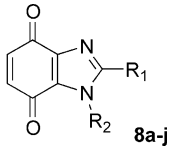
Nitration of 1,4-dimethoxybenzene (Scheme 1) afforded a mixture of 2,3-dinitro-1,4-dimethoxybenzene **1** and its 2,5-dinitro isomer **1'** in a ratio of 40/60, respectively (quantitative overall yield).

For the reduction of **1**, we obtained a better yield (98%) with Sn/HCl than with catalytic hydrogenation (Pd/C, MeOH/HCl). As starting from purified 2,3-dinitro compound **1** did not improve final yields, our sequence was carried out with the mixture of the two dinitro isomers.

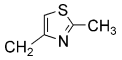
Treatment of the mixture **2** + **2'** with formic acid, acetic acid or phenylacetic acid in hydrochloric acid 6N led, respectively, to 4,7-dimethoxybenzimidazoles **3**, **4** and **5**. *N*-substituted compounds **6a–j** were obtained from **3–5** by treatment with NaH in DMF and subsequent addition of the suitable alkylating agent, except for **6g**. The latter was prepared by treatment of **3** with KOH in acetone following by addition of iodomethane.

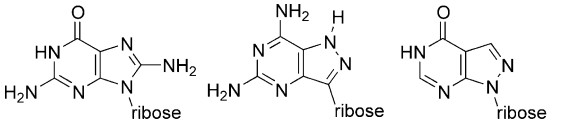
All our attempts to submit compound **6b** to an oxidative demethylation with ceric ammonium nitrate (CAN) or AgO led mainly to dimerization products and the final quinones were successfully obtained through a two-step process. Demethylation of **6a–j** with HBr provided **7a–j**. Oxidation of **7a** with aqueous $FeCl_3$ led to **8a**, whereas $K_2Cr_2O_7$ gave better results for *N*-substituted compounds **8b–j**.[12]

Table 1. Synthetized benzimidazole-4,7-diones **8a–j**



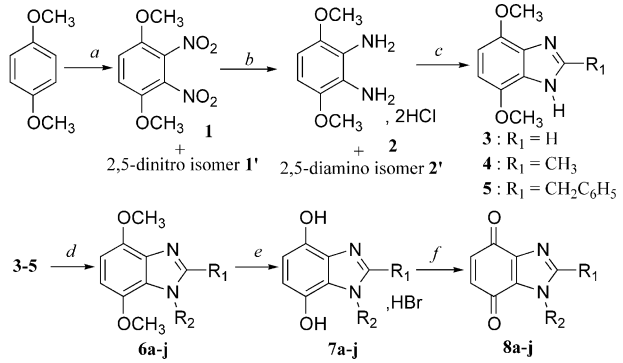
8a-j

Compd	R_1	R_2
8a[8]	H	H
8b	H	$CH_2C_6H_5$
8c	H	CH_2-*p*F-C_6H_4
8d	H	CH_2-*m*F-C_6H_4
8e	H	CH_2-*o*F-C_6H_4
8f	H	
8g[8]	H	CH_3
8h	H	*n*-C_4H_9
8i	CH_3	$CH_2C_6H_5$
8j	$CH_2C_6H_5$	$CH_2C_6H_5$



8-aminoguanosine (AG) **formycine A** (FoA) **allopurinol riboside** (AlloR)

Figure 1. Structure of the reference compounds.



Scheme 1. (a) HNO_3 70% 2 equiv/$(CH_3CO)_2O$, 0 °C, 3 h; (b) Sn/HCl 12 N, reflux, 2 h, (a) and (b) 98% overall yield; (c) R_1COOH, reflux, 5 h, 57–80% yield from pure **1**; (d) (1) NaH 1.05 equiv/DMF or KOH 5 equiv/CH_3COCH_3, rt; (2) R_2X. 0.95 equiv, rt, 12 h, 65–83% yield; (e) 48% aqueous HBr, 125°C, 5 h, 60–94% yield; (f) $FeCl_3$ 0.7 M, 30 min rt or $K_2Cr_2O_7$ 0.3 M/HCl 7 N, $HCCl_3$, 30 min, rt 68–95% yield.

Biology

Purine nucleoside phosphorylase was isolated from the RH and ME 49 strains of *T. gondii*. Both strains are maintained in culture with the human myelomonocytic cell line THP-1.[13] PNP was purified by the followings steps: precipitation with $(NH_4)_2SO_4$, DEAE-Sephacel and hydroxyapatite columns. The amount of the protein was determined by the Bensadoun's method.[14]

The drugs were tested on both PNP at the doses of 1 and 4 mM. The inhibition percentage of PNP specific activity[5] was defined as the ratio of PNP specific activity with drug on PNP specific activity without drug. K_i values were determined by using the Lineweaver–Burk method, with inosine as the variable substrate ranging from 0.5 to 3 mM and fixed inorganic phosphate concentration (50 mM).

Results and Discussion

8-Aminoguanosine (AG) was the first reported significant inhibitor of human erythrocyte PNP ($K_i = 7$ μM). We found (Table 2) that AG, albeit the most potent of the reference compounds, inhibits *T. gondii* PNP with a K_i of 200 μM, suggesting a marked difference in the active sites between *T. gondii* PNP and human PNP.

Furthermore, compounds **8** seem to have a slightly different behavior towards the enzymes of ME 49 strain and RH strain, indicating some differences between them.

All compounds **8a–j** possess some potency as PNP inhibitor but inhibit *T. gondii* PNP by different mechanisms. **8f** and **8i–j** are competitive inhibitors, while the others are noncompetitive.

It is noteworthy that **8i–j**, which are substituted at position 2, are among the most potent inhibitors of PNP from ME 49 strain, while the substitution of purine analogues at similar position, except with an amino group, leads generally to a marked decrease of the human PNP inhibition.[15,16]

Table 2. PNP inhibition of compounds **8a–j**

Compd	Inh (%) RH		K_i (mM)	Inh (%) ME49		K_i (mM)
	1 mM	4 mM	RH	1 mM	4 mM	ME 49
8a	47±1.4	68±1.4	1.65[a]	52±2.3	71±2.5	0.55[a]
8b	55±1.7	77±1.7	0.58[a]	57±3.0	89±3.0	0.67[a]
8c	80±2.4	88±1.8	0.78[a]	56±2.8	100±2.8	0.99[a]
8d	65±2.0	100±2	0.99[a]	60±2.0	100±2.0	0.52[a]
8e	70±2.1	87±1.7	1.48[a]	62±2.2	89±1.5	1.50[a]
8f	50±1.5	75±1.5	0.10[b]	44±3.0	77±2.1	0.11[b]
8g	55±1.7	85±1.7	2.21[a]	31±1.2	82±2.0	3.41[a]
8h	45±1.4	68±1.4	2.96[a]	53±2.2	65±2.0	0.56[a]
8i	54±1.6	82±1.6	1.05[b]	66±1.8	92±2.8	0.30[b]
8j	65±2.0	80±1.6	0.67[b]	61±3.2	78±2.3	0.35[b]
AG	85±2.6	91±2.7	0.20[b]	92±1.5	95±1.0	0.20[b]
FoA	12±0.4	37±1.1	1.68[b]	12±0.3	30±0.5	1.70[b]
AlloR	8±0.2	28±0.8	2.00[b]	11±0.2	22±0.5	1.80[b]

The values are the means±SD of triplicate experiments.
[a]Noncompetitive inhibition.
[b]Competitive inhibition.

In conclusion, *T. gondii* PNP represents a reasonable target for chemotherapy. Benzimidazole-4,7-diones are weak inhibitors of this enzyme, but some of them have shown a similar potency than 8-aminoguanosine, our best reference compound.

Further studies on new derivatives are at the present time in progress, as well as the evaluation of compounds **8** activity against *T. gondii* in culture.

Acknowledgements

The authors wish to thank Lyon I University (France) for financial support (BQR 2000). They are also grateful to Prof. H. Fillion (Lyon I University) for helpful discussions.

References and Notes

1. Tenter, A. M.; Heekeroth, A. R.; Weiss, L. M. *Int. J. Parasitol.* **2000**, *30*, 1217.
2. Scholer, N.; Krause, K.; Kayser, O.; Müller, R. H.; Borner, K.; Hahn, H.; Liesenfeld, O. *Antimicrob. Agents Chemother.* **2001**, *45*, 1771.
3. Hwang, H.-Y; Ullman, B. *J. Biol. Chem.* **1997**, *31*, 19488.
4. For a review on PNP, see: Bzowska, A.; Kulikowska, E.; Shugar, D. *Pharmacol. Ther.* **2000**, *88*, 349.
5. Ghérardi, A.; Sarciron, M.-E.; Pétavy, A.-F.; Peyron, F. *Life Sci.* **1999**, *65*, 1733.
6. (a) Schimandle, C. M.; Tanigoshi, L.; Mole, L. A.; Sherman, I. W. *J. Biol. Chem.* **1985**, *260*, 4455. (b) Bzowska, A.; Kulikowska, E.; Shugar, D. *Biochim. Biophys. Acta* **1992**, *1120*, 239. (c) Yokomatsu, T.; Hayakawa, Y.; Suemune, K.; Kihara, T.; Koyanagi, S.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem.* **2000**, *8*, 2571. (d) Evans, G. B.; Furneaux, R. H.; Gainsford, G. J.; Schramm, V. L.; Tyler, P. C. *Tetrahedron* **2000**, *56*, 3053. (e) Andricopulo, A. D.; Yunes, R. A. *Chem. Pharm. Bull.* **2001**, *49*, 10.
7. Pickering, M. V.; Dea, P.; Strater, D. G.; Witkowski, J. T. *J. Med. Chem.* **1977**, *20*, 818.
8. Zakhs, E. R.; Efros, L. S. *J. Org. Chem. U.S.S.R.* **1966**, 1089.
9. Daddona, P. E.; Wiesmann, W. P.; Milhouse, W.; Chern, J. W.; Townsend, L. B.; Hershfield, M. S.; Webster, H. K. *J. Biol. Chem.* **1986**, *261*, 11667.
10. Weinberger, L.; Day, A. R. *J. Org. Chem.* **1959**, *24*, 1451.
11. Grinev, A. N.; Zotova, A.; Bogdanova, N. S.; Nikolaeva, I. S.; Pershin, G. N. *Pharm. Chem. J.* **1974**, *8*, 135.
12. All new synthetized compounds gave satisfactory elemental analysis and were characterized by NMR spectroscopy.
13. Nebois, P.; Sarciron, M.-E.; Bibal, B.; Bouammali, B.; Cherkaoui, O.; Pautet, F.; Pétavy, A.-F.; Walchshofer, N.; Fillion, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 871.
14. Bensadoun; A; Weinstein, D. *Anal. Biochem.* **1976**, 241.
15. Jordan, F.; Wu, A. *J. Med. Chem.* **1978**, *21*, 877.
16. Farutin, V.; Masterson, L.; Andricopulo, A. D.; Cheng, J.; Riley, B.; Hakimi, R.; Frazer, J. W.; Cordes, E. H. *J. Med. Chem.* **1999**, *42*, 2422.