Bioorganic & Medicinal Chemistry Letters 20 (2010) 2938–2941

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A prodrug approach towards the development of tricyclic-based FBPase inhibitors

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ARTICLE INFO

Article history:
Received 25 December 2009
Revised 3 March 2010
Accepted 4 March 2010
Available online 7 March 2010

Keywords:
FBPase
Prodrug
CYP3A4

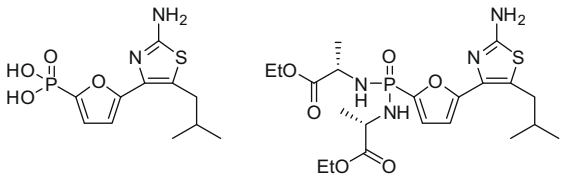
ABSTRACT

For the purpose of reducing the strong CYP3A4 inhibitory potency of diamide prodrug **4**, cyclic prodrugs of tricyclic-based FBPase inhibitors were synthesized. Extensive SAR studies led to the discovery of pyridine-containing cyclic prodrug **20**, which strongly inhibited glucose production in monkey hepatocytes and also showed weak CYP3A4 inhibitory potency.

Prodrug is a useful approach to overcoming a drug's defect including physiochemical, pharmacokinetic and pharmacodynamic properties.[1] Many kinds of prodrugs were designed to improve insufficient chemical stability, poor solubility or insufficient oral absorption. With the aim of improving insufficient oral absorption of compounds containing polar groups such as alcohols, carboxylic acids and phosphonic acids, a prodrug approach is frequently adopted to enhance membrane permeability. In particular, a phosphonate moiety possessing properties such as high ionization at physiologic pH and accompanying low membrane permeability requires a prodrug approach to obtain good oral bioavailability.[2]

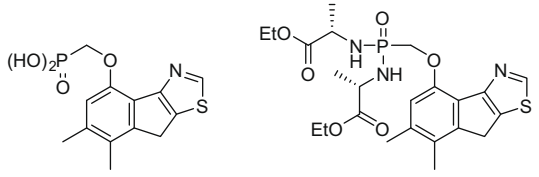
We have developed tricyclic-based phosphonates and corresponding prodrugs as fructose-1,6-bisphosphatase (FBPase) inhibitors.[3] FBPase inhibitors would lower blood glucose levels by reducing hepatic glucose output and are expected to be a novel class of drugs for the treatment of Type 2 diabetes mellitus. There are several small-molecule inhibitors of FBPase,[4–9] in which the prodrug of MB05032 (**1**) (CS-917, **2**) lowered blood glucose levels in animal models and was in clinical development (Fig. 1).[10] In previous papers, we reported that the prodrug of phosphonate **3** (**4**) strongly inhibited glucose production in hepatocytes and lowered blood glucose levels in fasted cynomolgus monkeys (Figure 2).[3c] However, subsequent tests revealed that diamide prodrug **4** strongly inhibited cytochrome P450 3A4 (CYP3A4).

In this Letter, we describe our efforts to solve the problem of the strong CYP3A4 inhibition of diamide prodrug **4**. CYP3A4 is responsible for metabolizing over 50% of drugs and the inhibition of



1 (MB05032) **2** (CS-917)

Figure 1. Structures of MB05032 and CS-917.



3 **4**

Figure 2. Structures of tricyclic-based FBPase inhibitors.

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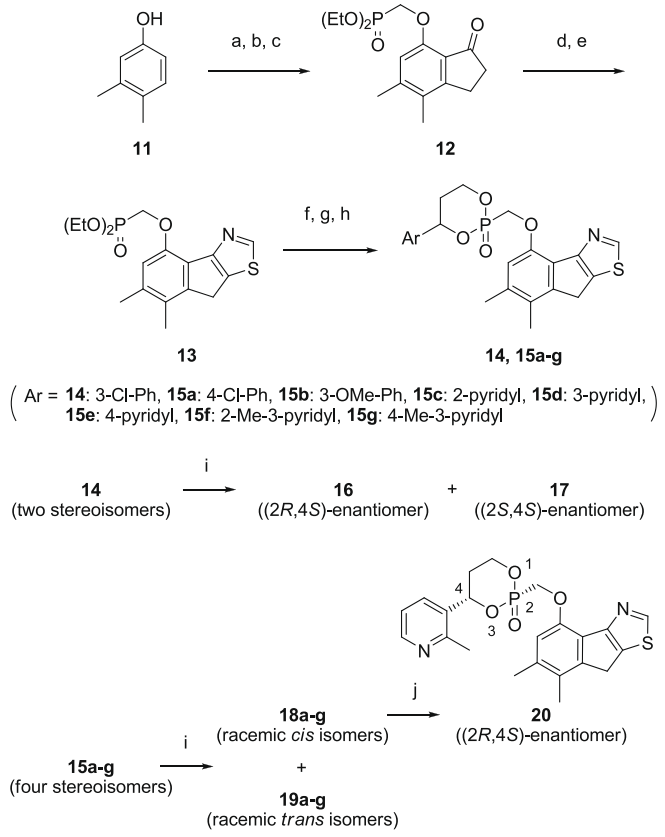
doi:10.1016/j.bmcl.2010.03.017

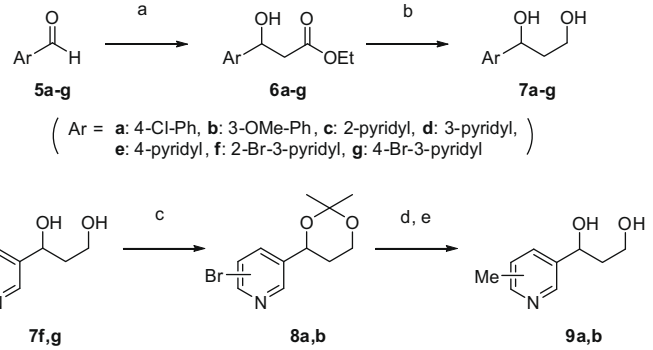
CYP3A4 is the major mechanism of drug–drug interactions, which can cause serious side effects in clinical practice. We expected that the conversion of the prodrug moiety of **4** to other prodrug moieties was beneficial in order to reduce the strong CYP3A4 inhibitory potency of **4**. Among many classes of phosphonate prodrugs, we are interested in cyclic 1-aryl-1,3-propanyl ester (HepDirect) prodrugs with respect to their application to pradefovir, which is in clinical trial for the therapy of hepatitis B infection.[11] This type of prodrug has an interesting property of being oxidatively cleaved by CYP in the liver, where our target enzyme FBPase is mainly expressed. We focused our attention on introducing various cyclic 1-aryl-1,3-propanyl ester moieties to the parent phosphonate **3** in order to reduce the strong CYP3A4 inhibitory potency of diamide prodrug **4**.

Initially, various 1-aryl-1,3-propanediols were synthesized according to Scheme 1. The condensation of ethyl acetate to the starting material aldehydes **5a–g** resulted in β-hydroxyesters **6a–g**, which were transformed into racemic diols **7a–g** by reduction with $LiAlH_4$. Pyridinediols containing a Me group (**9a,b**) were prepared from corresponding bromo-pyridinediols **7f,g** via ketalization, methylation and subsequent deketalization. In addition, (*S*)-1-(3-chlorophenyl)propane-1,3-diol **10** (prodrug moiety of pradefovir) was prepared following the literature method.[11a] Next, prodrugs **16–20** were prepared by the construction of tricyclic scaffold and subsequent introductions of prodrug moieties (Scheme 2). Acylation of 3,4-dimethyl phenol **11** followed by a Fries rearrangement and the introduction of a diethyl phosphonate unit resulted in diethyl phosphonate **12**, which was transformed into tricyclic thiazole **13** via bromination and cyclization with thioformamide (in situ generation). Cleaving phosphonate ethyl groups of **13** followed by dichlorination and subsequent condensation with corresponding chiral diol **10** or racemic diols **7a–e**, **9a,b** afforded cyclic prodrugs **14** (mixtures of two stereoisomers) or **15a–g** (mixtures of four stereoisomers), respectively. Enantiomers **16** and **17** were obtained by silica gel separation of diastereomeric mixture **14**. In addition, the facile separation of four stereoisomers **15a–g** with silica gel chromatography led to racemic *cis* isomers **18a–g** and racemic *trans* isomers **19a–g**. Moreover, the chiral HPLC separation of racemic *cis* isomer **18f** afforded (2*R*,4*S*)-enantiomer **20**, the structure of which was determined by single-crystal X-ray analysis (Fig. 3).

Prodrugs containing a benzene ring showed strong CYP3A4 inhibitory potencies and poor aqueous solubilities (Table 1). Initially, the prodrug moiety of pradefovir was introduced to our tricyclic phosphonate. The (2*R*,4*S*)-enantiomer **16** inhibited glucose production in hepatocytes (IC_{50} = 15 μM), but also showed a strong CYP3A4 inhibitory potency (IC_{50} = 0.42 μM) almost equal to that of diamide prodrug **4** (IC_{50} = 0.58 μM). Next, other substituted benzene-containing prodrugs, which were prepared as racemic *cis* and racemic *trans* isomers, were evaluated. Only the *cis* isomer of 4-chloro substituted analogue (**18a**) modestly inhibited glucose production in hepatocytes (IC_{50} = 66 μM), whereas other prodrugs including 3-methoxy analogues showed no inhibitory activities. In addition, these prodrugs showed strong CYP3A4 inhibitory potencies and poor aqueous solubilities. It is known that the potency of CYP3A4 inhibition correlates with compound lipophilicity.[12] We thought that decreasing lipophilicity of our prodrugs was beneficial in order to reduce the potency of CYP3A4 inhibition and also to improve aqueous solubility, which prompted us to introduce a pyridine ring to the prodrug instead of a benzene ring.



Scheme 2. Synthesis of prodrugs **16**–**20**. Reagents and conditions: (a) $ClCH_2CH_2COCl$, pyridine, CH_2Cl_2, 72%; (b) $AlCl_3$, 180 °C, 78%; (c) $(EtO)_2P(O)CH_2OTs$, K_2CO_3, DMF, 80 °C, 78%; (d) $CuBr_2$, EtOH, 60 °C, 98%; (e) P_2S_5, $HCONH_2$, THF, reflux, 58%; (f) TMSBr, CH_2Cl_2; (g) $(COCl)_2$, DMF, CH_2Cl_2; (h) diol, DIPEA, CH_2Cl_2, 0 °C, 19–61% over three steps; (i) silica gel separation; (j) chiral HPLC separation (CHIRALPAK AD-H).



Scheme 1. Synthesis of diols **7**, **9**. Reagents and conditions: (a) $^{i}Pr_2NH$, *n*BuLi, EtOAc, THF, −78 °C to rt; (b) $LiAlH_4$, THF, 0 °C, 17–94% over two steps; (c) $Me_2C(OMe)_2$, TsOH, acetone, reflux, 62–85%; (d) *n*BuLi, MeI, THF, −78 °C to rt; (e) HCl_{aq}, MeOH, 39–79%, over two steps.

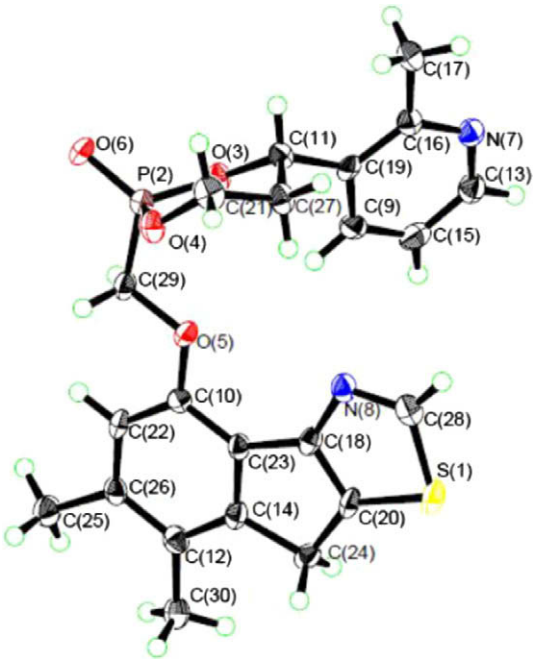
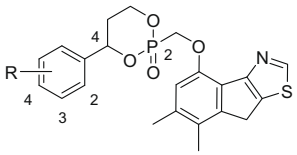


Figure 3. ORTEP view of prodrug **20** from single-crystal X-ray analysis.

Prodrugs containing the pyridine ring demonstrated improved results compared with prodrugs containing the benzene ring (Table 2). Although both racemic *cis* and racemic *trans* isomers of pyridine-containing prodrugs were evaluated, racemic *trans* isomers **19c–g** did not inhibit glucose production in hepatocytes (data not shown). The efforts to incorporate unsubstituted pyridine rings (**18c**, **18d**, **18e**) led to a decrease of CYP3A4 inhibitory potencies and an improvement in aqueous solubility relative to benzene-containing prodrugs. Among these unsubstituted pyridine analogues, 3-pyridyl analogue **18d** most potently inhibited glucose production in hepatocytes (IC_{50} = 8.8 μM). Introducing the methyl group into *ortho* positions of the pyridine nitrogen showed opposite effects on the inhibition of glucose production in hepatocytes, including little effect with the 2-Me group (**18f**, IC_{50} = 11 μM) and a detrimental effect with the 4-Me group (**18g**, IC_{50} = >100 μM). These methyl-substituted pyridine analogues **18f** and **18g** showed weak CYP3A4 inhibitory potencies (IC_{50} = 5.8, 5.2 μM, respectively) compared with the corresponding unsubstituted pyridine analogue **18d** (IC_{50} = 2.9 μM), presumably due to the steric effects on pyridine nitrogen, which is supposed to bind to the CYP heme iron. Moreover, (2*R*,4*S*)-enantiomer of **18f** (**20**) more strongly inhibited glucose production in hepatocytes (IC_{50} = 4.0 μM) and showed weak CYP3A4 inhibitory potency (IC_{50} = 15 μM) relative to **18f**. Compared with diamide prodrug **4**, pyridine-containing cyclic prodrug **20** more potently inhibited glucose production in hepatocytes, and showed an over 25 times weaker CYP3A4 inhibitory potency. These results revealed that prodrug **20** would have a reduced risk of problematic drug–drug interactions.

Pyridine-containing prodrug **20** appears to have more favorable physicochemical properties than other types of prodrugs (Table 3). In a previous paper, we revealed the importance of the physico-

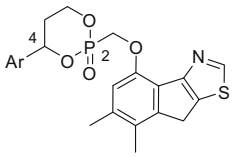
Table 1
SAR of prodrugs containing a benzene ring[13]



Compound	R	P2, C4 stereochemistry	Hepatocytes	CYP3A4	Solubility (μg/ml)	
			IC_{50}[a] (μM)	IC_{50} (μM)	JP1 (pH 1.2)	JP2 (pH 6.8)
4	—	—	6.8	0.58	27	66
16	3-Cl	*cis*-(2*R*,4*S*)	15	0.42	19	<0.5
17	3-Cl	*trans*-(2*S*,4*S*)	>100	2.7	<0.5	<0.5
18a	4-Cl	Racemic *cis*	66	0.65	4.4	<0.5
19a	4-Cl	Racemic *trans*	>100	0.73	<0.5	<0.5
18b	3-OMe	Racemic *cis*	>100	0.27	17	0.8
19b	3-OMe	Racemic *trans*	>100	0.3	<0.5	<0.5

[a] Inhibition of glucose production in primary monkey hepatocytes.

Table 2
SAR of prodrugs containing a pyridine ring[13]



Compound	Ar	P2, C4 stereochemistry	Hepatocytes	CYP3A4	Solubility (μg/ml)	
			IC_{50}[a] (μM)	IC_{50} (μM)	JP1 (pH 1.2)	JP2 (pH 6.8)
18c	2-Py	Racemic *cis*	22	8.4	86	73
18d	3-Py	Racemic *cis*	8.8	2.9	86	84
18e	4-Py	Racemic *cis*	41	2.2	88	51
18f	2-Me-3-Py	Racemic *cis*	11	5.8	87	83
18g	4-Me-3-Py	Racemic *cis*	>100	5.2	>100	81
20	2-Me-3-Py	*cis*-(2*R*,4*S*)	4.0	15	88	84

[a] Inhibition of glucose production in primary monkey hepatocytes.

Table 3
Physicochemical properties of prodrugs[a]

Compound	MW	HB-D[b]	HB-A[c]	c Log *P*	PSA[d]	Hepatocytes	CYP3A4	Solubility (μg/ml)[f]	
						IC_{50}[e] (μM)	IC_{50}[f] (μM)	JP1 (pH 1.2)	JP2 (pH 6.8)
4	509.56	2	9	2.93	153.9	6.8	0.58	27	66
16	461.90	0	5	4.39	95.7	15	0.42	19	<0.5
20	442.47	0	6	2.76	108.59	4.0	15	88	84

[a] Calculated using ACD/labs version 9.0 (Advanced Chemistry Development, Inc.).
[b] Hydrogen bond donors.
[c] Hydrogen bond acceptors.
[d] Polar surface area (Å^2).
[e] Inhibition of glucose production in primary monkey hepatocytes (experimental values).
[f] Experimental values.

chemical parameter of prodrugs and also suggested that the low PSA values related to high membrane permeability of the prodrugs was probably critical to the strong inhibitory effect on glucose production in hepatocytes.[3c] With reference to physicochemical parameters of cyclic prodrugs, it is apparent that benzene-containing prodrug **16** has a high *c* Log *P* value (4.39), which perhaps resulted in the strong CYP3A4 inhibitory potency and poor aqueous solubility of **16**. Compared with benzene-containing prodrug **16**, pyridine-containing prodrug **20** has a low *c* Log *P* value (2.76), which probably led to the weak CYP3A4 inhibitory potency and improved aqueous solubility of **20**. Furthermore, the low PSA value of pyridine-containing prodrug **20** (108.59) relative to that of diamide prodrug **4** (153.9) presumably contributed to the strong inhibitory effect on glucose production in hepatocytes.

In summary, we developed cyclic prodrugs of tricyclic-based FBPase inhibitors in order to reduce the strong CYP3A4 inhibitory potency of diamide prodrug **4**. In contrast to prodrugs containing a benzene ring, prodrugs containing a pyridine ring showed weak CYP3A4 inhibitory potency and high aqueous solubility, which was presumably linked to low *c* Log *P* values of the prodrugs. Pyridine-containing cyclic prodrug **20** more potently inhibited glucose production in hepatocytes and also showed weaker CYP3A4 inhibitory potency than diamide prodrug **4**. These results not only revealed that prodrug **20** would have a reduced risk of problematic drug–drug interactions, but also demonstrated the importance of optimizing a prodrug to improve a drug's defect.

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13. (a) For details of assays to measure the inhibitory effects on glucose production in monkey hepatocytes, see Ref. 3c; (b) The potency of test compounds to inhibit expressed CYP3A4 (Supersomes, BD Gentest, MA, USA) was measured using 7-benzyloxy-4-trifluoromethylcoumarin as substrate; (c) The solubility of test compounds was assayed using the first (JP1, pH 1.2) and second (JP2, pH 6.8) fluids of Japanese Pharmacopoeia, which simulate gastric and intestinal pH conditions, respectively.