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Novel prodrugs with a spontaneous cleavable guanidine moiety

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ABSTRACT

Water-soluble prodrug strategy is a practical alternative for improving the drug bioavailability of sparingly-soluble drugs with reduced drug efficacy. Many water-soluble prodrugs of sparingly-soluble drugs, such as the phosphate ester of a drug, have been reported. Recently, we described a novel water-soluble prodrug based on *O–N* intramolecular acyl migration. However, these prodrug approaches require a hydroxy group in the structure of their drugs, and other prodrug approaches are often restricted by the structure of the parent drugs. To develop prodrugs with no restriction in the structure, we focused on a decomposition reaction of arginine methyl ester. This reaction proceeds at room temperature under neutral conditions, and we applied this reaction to the prodrug strategy for drugs with an amino group. We designed and synthesized novel prodrugs of representative sparingly soluble drugs phenytoin and sulfathiazole. Phenytoin and sulfathiazole were obtained as stable salt that were converted to parent drugs under physiological conditions. Phenytoin prodrug **3** showed a short half-life ($t_{1/2}$) of 13 min, whereas sulfathiazole prodrug **7** had a moderate $t_{1/2}$ of 40 min. Prodrugs **3** and **7** appear to be suitable for use as an injectable formulation and orally administered drug, respectively.

Prodrug strategy is a practical alternative for improving drug bioavailability, since it overcomes various barriers that reduce drug efficacy, including poor solubility. There are many well-known sparingly-soluble drugs, and they often exhibit reduced bioavailability and have undesirable pharmaceutical properties.[1–3] For example, most human immunodeficiency virus type 1 (HIV-1) protease inhibitors are poorly soluble in aqueous solutions, such as gastric juice. HIV-1 protease is a dimer, and the pockets around its active site are hydrophobic. Thus, potent inhibitors that are optimized for the pockets of HIV-1 protease show poor water-solubility. The formulations of HIV-1 protease inhibitors, such as ritonavir, contain some solubilizers, such as polyoxyhydrogenated caster oils, which lead to some side effects. The antiepileptic phenytoin is a representative sparingly-soluble drug. Because phenytoin is only dissolved in strongly-alkaline aqueous solutions, the injectable formulations of phenytoin are prepared as an aqueous solution at pH 12, often resulting in strong irritant properties. Recently, we reported a series of β-secretase (β-site amyloid precursor protein cleaving enzyme 1; BACE1) inhibitors as anti-Alzheimer's disease drugs.[4–16] Because BACE1 also has some hydrophobic pockets around its active site, BACE1 inhibitors optimized for the hydrophobic pockets were often sparingly-soluble. To improve the poor solubility, one effective strategy is to convert the poor-soluble drugs into hydrophilic prodrugs by covalently attaching the appropriate solubilizing moieties, such as phosphates,[17,18] sugars,[19,20] and amines,[21,22] which can eliminate from the prodrugs enzymatically or chemically under physiological conditions. The hydroxy groups in drugs are often modified for the application to prodrugs because they are easily cleaved. The phosphate-type water-soluble prodrugs of HIV-1 protease inhibitors were reported by Taisrivongs et al.[23] These prodrugs can release the parent drugs by cleaving a phosphate ester bond through the help of an alkaline phosphatase. Thus, this type of prodrug requires a hydroxy group in the structure of the parent drug. Previously, we reported novel water-soluble prodrugs based on *O–N* intramolecular acyl migration reaction.[24–28] These prodrugs, wherein the amide bond of the parent drug is converted to an ester bond, could be rapidly converted to the corresponding parent drugs under physiological conditions. However, there is a structural restriction for the application of these prodrugs, in particular, as the parent drugs must have a hydroxy group in their structure. Moreover, our water-soluble ritonavir prodrug exhibited a drug release rate that was too slow ($t_{1/2}$ = 42 h).[24] The prodrugs with a steric hindered hydroxy group, such as ritonavir, might have slow drug release rates because *O–N* intramolecular acyl migration requires a conformational change for the formation of a transition state that accompanies the intramolecular migration reaction. As seen above, there are various structural restrictions for the design of water-soluble prodrugs. New prodrug strategies are required with no

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structural restriction and with a more rapid drug release rate and spread wide modifiable functional groups, such as an amino group, other than hydroxy group.

To overcome these issues, we focused on a decomposition reaction of arginine methyl ester, which was reported by Photaki et al.,[29] as shown in Figure 1A. In this reaction, the guanidino group of an arginine methyl ester attacks the ester's carbonyl carbon of another arginine methyl ester, forming the arginine dimer. Next, N-terminal amino group of the dimer attack the guanidine's carbon within the molecule, forming a heterocyclic compound and ornithine methyl ester. Surprisingly, this decomposition reaction proceeds at room temperature under neutral conditions. Hence, we believe that the second reaction in the decomposition of arginine methyl ester can be applied to prodrug design. We focused on the aminoacylguanidine structure of the arginine dimer and designed a novel prodrug (Type A), as shown in Fig. 1B. This prodrug is thought to release a heterocyclic compound—glycocyamidine—forming the parent drug. We assumed that a driving force of this reaction was the release of the heterocyclic compound that was stabilized with a conjugate structure. Because a compound in which the order of the acyl and guanidino group of the prodrug (Type A) is reversed is thought to release the same heterocyclic compound to the prodrug (Type A), we speculated that the release of the heterocyclic compound might become a driving force for the cleavage reaction of an amide bond and designed another novel prodrug (Type B), as shown in Figure 1B.

First, we elected 2-aminobenzimidazole as a model compound for evaluating our prodrug approach, because many sparingly-soluble drugs have a/some amino group(s) on a heterocyclic ring or aromatic ring, whereas drugs with an aliphatic amino group are easily-soluble as a salt in aqueous solutions. We designed and synthesized two types of prodrugs (**1** and **2**) of 2-aminobenzimidazole, as shown in Figure 2. Subsequently, we synthesized the prodrugs **2**–**7** of the representative sparingly-soluble drugs, phenytoin and sulfathiazole, and also the prodrugs **8** and **9** of the anti-malarial quinine with a hydroxy group.

Compounds **1**–**9** were synthesized using the common solution-phase synthesis method as shown in Scheme 1. Amide and ester bond formation for preparing **10** and **11a**–**e** was performed using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC) in the presence of 4-dimethylaminopyridine (DMAP) as a coupling reagent. Guanidination reaction for preparing **13a**–**e** was performed using *N*,*N*′-Bis(tert-butoxycarbonyl)1*H*-pyrazole-1-carboxamidine in acetonitrile or acetonitrile containing a small amount of dimethylformamide (DMF). The guanidination reaction generally proceeded more rapidly in low polar solvents. Because most

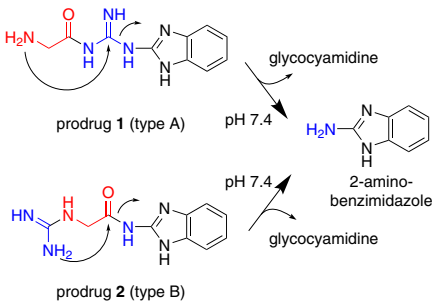


Figure 2. Design of two kinds of 2-aminobenzimidazole prodrugs.

materials are poorly soluble in low polar solvents, we used acetonitrile with/without DMF for the guanidination reaction. Boc-deprotection of compounds **11a**–**e** was performed using anisole and 4 N HCl/dioxane, whereas Boc-protection for preparing **1**–**9** was performed using trifluoroacetic acid (TFA). Prodrugs **1**–**9** were obtained as TFA salts and stored in a refrigerator.

Prodrugs **1**–**9** were evaluated by high-performance liquid chromatography (HPLC) analysis using a reverse phase C18 column and a linear gradient system of acetonitrile and 0.1% aqueous TFA. These prodrugs and peptide were incubated under physiological condition with pH 7.4 phosphate buffered saline (PBS) at 37 °C and measured by HPLC. Prodrug **1** was stable as a TFA salt, but **1** was labile in polar solvents, such as methanol and aqueous solvents, and released quickly the parent compound 2-aminobenzimidazole. Thus, Type A prodrugs appeared to be not appropriate for the prodrug strategy. Prodrug **2** (Type B) was obtained as a TFA salt that was stable in acidic media, methanol, and unbuffered water. Prodrug **2** (Type B) appeared to be present as a TFA salt in methanol and unbuffered water. HPLC profile and time course of **2** in pH 7.4 PBS at 37 °C are shown in Figure 3A and B, respectively. Prodrug **2** released time-dependently ($t_{1/2}$ value: 38 min, Table 1) its parent drug 2-aminobenzimidazole with no byproduct.

Based on these results, we synthesized a series of Type B prodrugs (**2**–**9**) of phenytoin, sulfathiazole, and quinine. Although the NH group of phenytoin is not an amino group but an imide group, the prodrugs (**3**–**5**) of representative sparingly-soluble drug, phenytoin, were synthesized first. Prodrug **3** rapidly converted to the parent drug, phenytoin, in a time-dependent manner with no byproduct. The phenytoin prodrug Fostoin (fosphenytoin), which possesses a phosphate ester bond, was approved by the US Food and Drug Administration in 2004 for injection. Fostoin releases the parent drug, phenytoin, and formaldehyde in the presence of

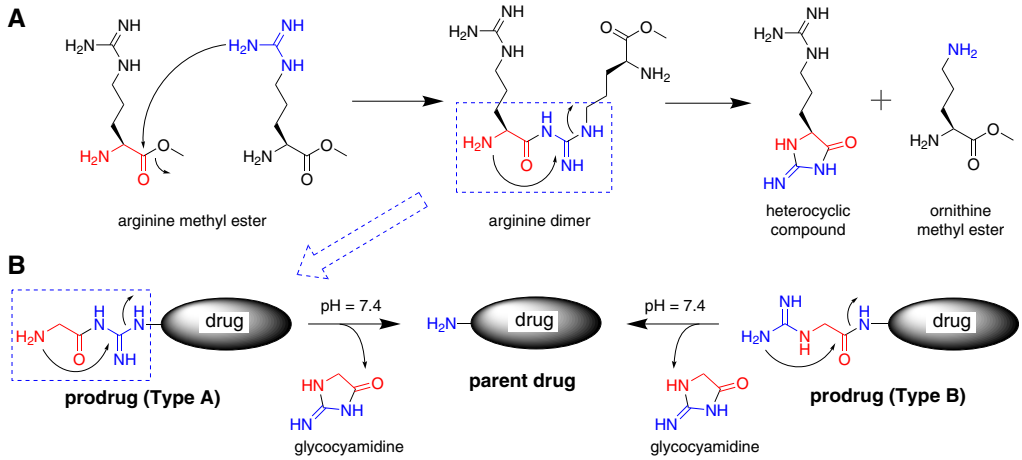
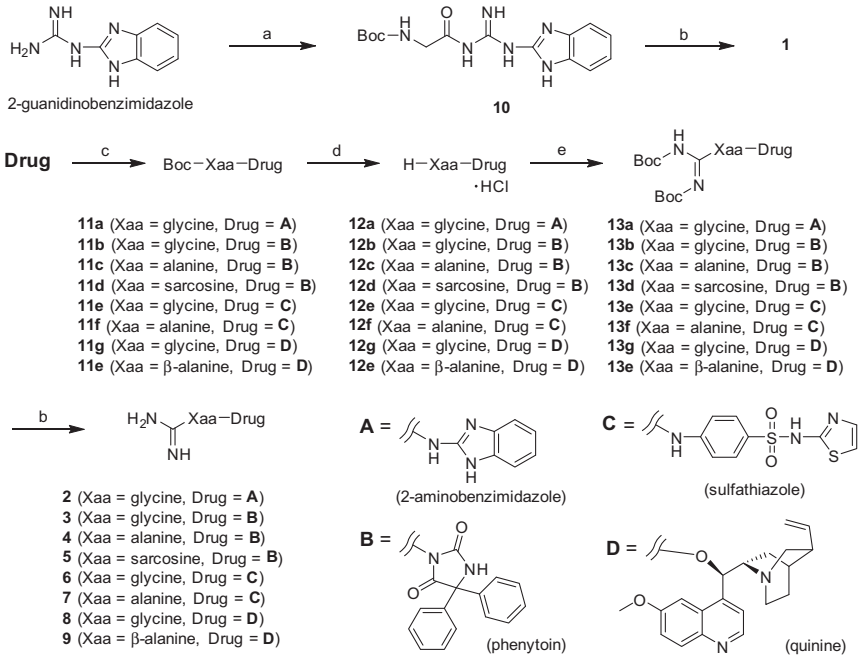


Figure 1. (A) Decomposition reaction of arginine methyl ester. (B) Design of two types of prodrugs based on the decomposition reaction of arginine methyl ester.



Scheme 1. Reagents and condition: (a) Boc-Gly-OH, EDC, DMAP/DMF, room temperature, 1 day; (b) TFA, room temperature, 2 h; (c) Boc-Gly-OH, Boc-Ala-OH, or Boc-Sar-OH, EDC, DMAP/DMF, room temperature, 1 day; (d) anisole, 4 M HCl/dioxane, room temperature, 2 h; (e) *N*,*N*-Bis(tert-butoxycarbonl)1H-pyrazole-1-carboxamidine, acetonitrile, room temperature, 1 day.

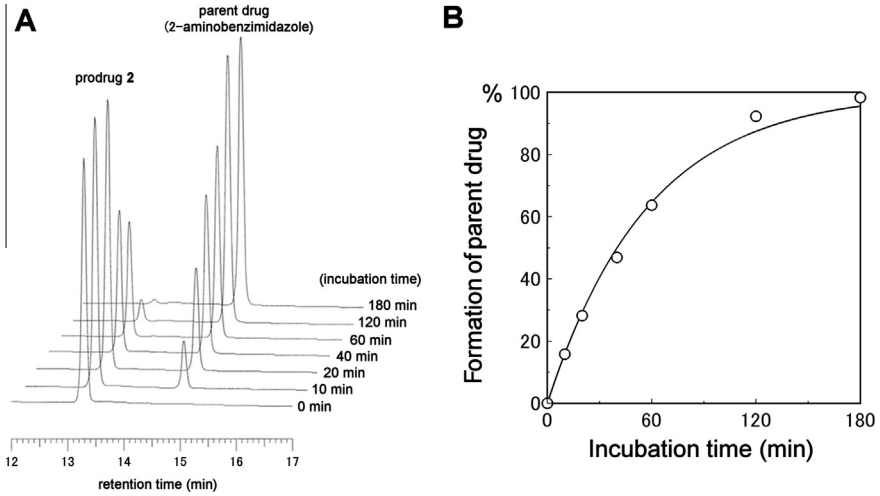
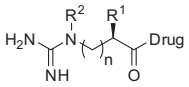


Figure 3. (A) HPLC profile of prodrug **2** in pH 7.4 PBS at 37 °C. (B) Formation rate of 2-aminobenzimidazole from prodrug **2** in pH 7.4 PBS at 37 °C.

an alkaline phosphatase, and shows a $t_{1/2}$ value of 8 min in human plasma, which is close to that of prodrug **3** ($t_{1/2}$ = 13 min).[30] Prodrug **3** appears to be interchangeable with Fostoin for clinical use and is therefore suitable as an injectable formulation. Prodrug **4** and **5**, which possess an α-methyl or *N*-methyl group in the amino acid moiety, were converted more rapidly to the parent drug than prodrug **3**. As it is well-known that a/some methyl group(s) on a leaving group accelerate the cyclization reaction via nucleophile attack,[31] this result seemes predictable. The sulfa drug sulfathiazole is a representative of a sparingly-soluble drug and has a typical aromatic amino group. Prodrug **6** of sulfathiazole was synthesized, but **6** and its parent drug sulfathiazole had the same retention time on HPLC, and the drug release rate of **6** could not be evaluated. Therefore, we synthesized prodrug **7**, which possesses an α-methyl group in the amino acid moiety. Prodrug **7** was converted to the parent drug in a time dependent manner, and it showed a moderate drug release rate ($t_{1/2}$ value; 40 min) and appeared to be suitable as an orally-administered drug.

To demonstrate the wide application of our prodrug strategy, prodrug **8** of quinine with a hydroxy group was synthesized. As an ester bond is more cleavable than an amide bond, the drug release rate of prodrug **8** was more rapid than that of amide-type prodrugs **2**–**7**. We previously reported that ester-type prodrugs based on *O*–*N* intramolecular acyl migration via formation of a six-membered ring showed slower drug release rates than the same type prodrugs that formed a five-membered ring.[25] Because the drug release rate of prodrug **8** was extremely rapid ($t_{1/2}$ value; <1 min, Table 1), prodrug **9** was synthesized via formation of a six-membered ring to delay the drug release rate. Prodrug **9** was rapidly converted to the parent drug in a time-dependent manner ($t_{1/2}$ value; 1.1 min, Table 1) with no byproduct (see Supporting information) as shown in Figure 4.

Table 1
Drug release rate and solubility of prodrugs



Prodrug	Parent drug	R^1	R^2	n	$t_{1/2}$ value[a] (min)	Solubility (mg/mL)		Ratio of solubility[b]
						Parent drug[c]	Prodrug	
2	2-Aminobenzimidazole	–H	–H	0	38	–	–	–
3	Phenytoin	–H	–H	0	13	6.3×10^{-3}	10.2	1620
4	Phenytoin	$-CH_3$	–H	0	1.1	–	–	–
5	Phenytoin	–H	$-CH_3$	0	0.78	–	–	–
7	Sulfathiazole	$-CH_3$	–H	0	40	1.4×10^{-3}	7.1	5071
8	Quinine	–H	–H	0	<1	–	–	–
9	Quinine	–H	–H	1	1.1	2.1×10^{-1}	>10	>48

[a] $t_{1/2}$ is the time required for 50% release of parent drugs in pH 7.4 PBS at 37 °C.
[b] Prodrug/parent drug.
[c] The parent drugs, sulfathiazole and quinine, were used as a TFA salt and an HCl salt, respectively, for measuring the solubilities.

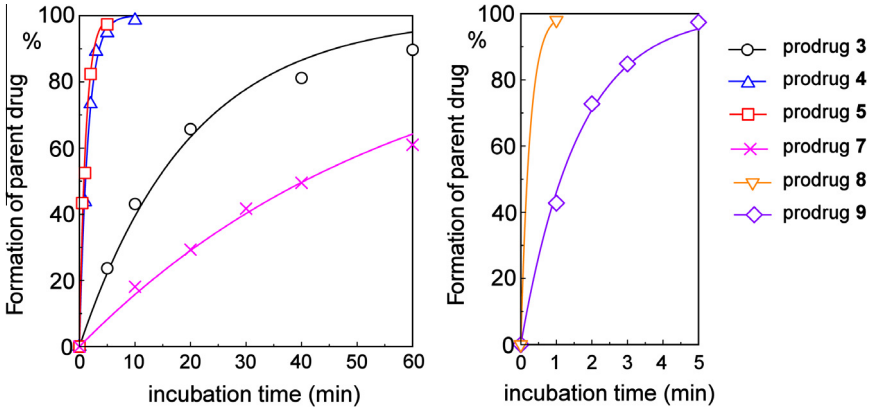
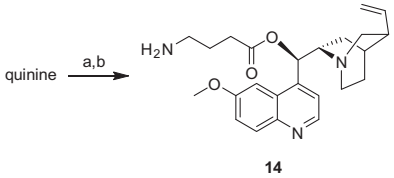


Figure 4. Formation of parent drugs from phenytoin prodrugs (**3**–**5**), sulfathiazole prodrug (**7**), and quinine prodrugs (**8** and **9**) in pH 7.4 PBS at 37 °C.

The water-solubility and stability of synthetic prodrugs **3**, **7**, and **9** was evaluated using HPLC analysis (see Supporting information). In all cases, the tested prodrugs exhibited water-solubility better than that of the corresponding parent drugs (Table 1). Prodrugs **3**, **7**, and **9** were stable as TFA salts for 1 month. Although storage of prodrugs **3**, **7**, and **9** in physiological saline solution for 1 day resulted in 4%, 0%, and 6% drug release, respectively, such release does not seem to present an obstacle to clinical use of the prodrugs as injectable formulations.

Although the cleavage of an amide bond is difficult in the absence of an enzyme, many ester-type prodrugs are known. A conventional ester-type prodrug of quinine, **14**, was synthesized for comparison with our ester-type prodrug of quinine **8**. Prodrug **14** was synthesized using a similar method as that of prodrugs **2**–**9**, as shown in Scheme 2. Prodrug **14** with a γ-aminobutyric group was obtained as a TFA salt, and seems to exist as a TFA salt in unbuffered aqueous solutions, such as water. Prodrug **14** releases the parent drug quinine and a five-membered heterocyclic compound derived from the γ-aminobutyric group as shown in Figure 5. Although prodrug **14** released its parent drug quinine in a time-dependent manner with no byproduct, the rate was very slow ($t_{1/2}$ value: 1.7 h, Figure 6) in pH 7.4 PBS at 37 °C. Most of the well-known ester-type prodrugs require the presence of enzymes, such as alkaline phosphatase and esterase.[32] No spontaneously cleavable ester-type prodrugs have been used in clinical practice owing to the slow drug release rate. Although prodrug **14** ($t_{1/2}$ = 1.7 h) forms the same five-membered ring as **8** ($t_{1/2}$ <1 min), the $t_{1/2}$ value of **14** was more than hundred-fold of that of **8**. This result appears to demonstrate the advantage of our prodrug strategy.

In conclusion, we designed and synthesized a series of novel water-soluble prodrugs based on a decomposition reaction of arginine methyl ester. These prodrugs could be converted to the respective parent drugs between the $t_{1/2}$ values of 1 min and



Scheme 2. Reagents and condition: (a) Boc-NH$(CH_2)_3$COOH, EDC, DMAP/DMF, room temperature, 1 day; (b) TFA, room temperature, 2 h.

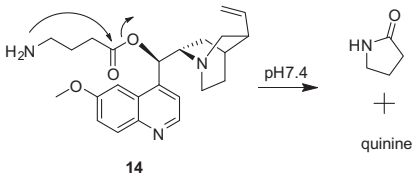


Figure 5. Conventional ester-type prodrug of quinine.

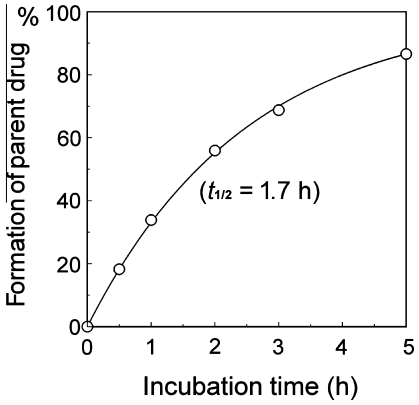


Figure 6. Formation rate of quinine from conventional ester-type prodrug of quinine, 14, in pH 7.4 PBS at 37 °C.

40 min. Phenytoin prodrug **3** ($t_{1/2}$ value; 13 min) and sulfathiazole prodrug **7** ($t_{1/2}$ value; 40 min) are suitable for an injectable formulation and an orally-administered drug, respectively. Although the $t_{1/2}$ of quinine prodrug **8** was too short to allow clinical use of the compound (<1 min), quinine prodrug **9** had a longer $t_{1/2}$ of 1.1 min, demonstrating that alteration of the guanidino-acyl moiety altered the pharmacokinetic characteristics of these prodrugs. These results suggest that a quinine prodrug with a longer and clinically suitable $t_{1/2}$ can be potentially developed by utilizing our prodrug strategy. Malaria is a disease caused by parasitic protozoa of the genus *Plasmodium*, which are transmitted to humans by the *Anopheles* mosquito. Malaria is a potent threat to human beings; indeed, the range of the *Anopheles* mosquito is expanding as a result of global warming. Recently, chloroquine-resistant strains of *Plasmodium* protozoa have been identified, while the use of quinine has increased. Patients with severe malaria require intravenous administration of quinine for a period of 4 h. If the current quinine treatment regimen can be modified to consist of a single injection through the use of our prodrug approach, the burden of malaria on many patients might be reduced.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.060.

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