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The synthesis of amphipathic prodrugs of 1,2-diol drugs with saccharide conjugates by high regioselective enzymatic protocol

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Abstract—A facile, high regioselective enzymatic synthesis approach for the preparation of amphipathic prodrugs with saccharides of mephenesin and chlorphenesin was developed. Firstly, transesterification of two drugs with divinyl dicarboxylates with different carbon chain length was performed under the catalysis of *Candida antarctica* lipase acrylic resin and Lipozyme® in anhydrous acetone at 50 °C, respectively. A series of lipophilic derivatives with vinyl groups of mephenesin and chlorphenesin were prepared. The influences of different organic solvents, enzyme sources, reaction time, and the acylation reagents on the synthesis of vinyl esters were investigated. And then, protease-catalyzed high regioselective acylation of D-glucose and D-mannose with vinyl esters of mephenesin and chlorphenesin gave drug–saccharide derivatives in good yields. The studies of lipophilicity and hydrolysis in vitro of prodrugs verified that drug–saccharide derivatives had amphipathic properties, and both lipophilic and amphipathic drug derivatives had obvious controlled release characteristics.

1. Introduction

Amphipathic prodrugs are one of the most effective surface-active drugs and attracting much interest in recent years. They can obviously affect the absorption of drugs through cell membranes and biological barriers. In particular, it would like to self-assemble into ordered aggregates such as micelles and vesicles to deliver themselves in vivo.[1–4] For example, many stable non-covalent drug-surfactant conjugates are amphipathic, and generally used to enhance the permeability of cell membranes.[5–7] Some amphipathic macromolecule drugs are designed for improving pharmaceuticals delivery properties and their therapeutic effect.[8,9] Saccharides are highly hydrophilic and play an important role in biological recognition processes. A great number of drugs in use today have relied on carbohydrates for their therapeutic action. The incorporation of carbohydrates offers many opportunities for preparing amphipathic prodrugs with bioactive moieties and spans a wide range of drug types.[10]

Glycoconjugates of drugs contain a large number of functional groups that are nearly indistinguishable chemically. However, it is well known that the derivations of drugs at specific group have clear therapeutic advantages, including high activity and reduced toxicity.[11] Therefore, it is crucial to have good selectivity in synthesis procedure. Enzyme-catalyzed reactions are widely recognized as superior to conventional chemical methods in selective modification of polyfunctional substrates owing to mild reaction conditions, high catalytic efficiency, inherent selectivity, and simple downstream processing. Therefore, many research groups have paid much effort in the area of enzymatic synthesis of drug derivatives.[12–16]

In pursuit to synthesize amphipathic small molecule prodrugs with sugar moieties, we have selected mephenesin and chlorphenesin as substrates. As one type of 1,2-diol drugs, they have centrally active muscle relaxant effect and sedative properties. In addition, mephenesin is effectively antitoxic agent for deltamethrin.[17–20] Chlorphenesin is also used to treat injuries and other painful muscular conditions along with rest and physical therapy.[21–23] The two drugs exhibit a short half-life in the blood stream and a high overall clearance rate. Amphipathic glycoconjugates of mephenesin and chlorphenesin maybe overcome these disadvantages and possess better therapeutic effect.

Keywords: Amphipathic prodrug; Enzymatic synthesis; Mephenesin; Chlorphenesin.
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Herein, we have developed a facile and high regioselective enzymatic method to prepare amphipathic drug–saccharide derivatives. The lipophilicity and in vitro hydrolysis of prodrugs were investigated. Results showed that the derivatives of mephenesin and chlorphenesin containing sugar moiety had amphipathic characteristics, which would like to permeate biomembranes and well solubilize in water. And then both lipophilic and amphipathic derivatives had obvious sustained release characteristics compared with their parent drugs. These characteristics will have potential value for clinic symptom. After optimizing the reaction conditions, fast reaction rate and good yields were achieved.

2. Results and discussion

2.1. Enzymatic synthesis of drug vinyl esters

Transesterifications of mephenesin and chlorphenesin with divinyl dicarboxylates catalyzed by *Candida antarctica* lipase acrylic resin and Lipozyme®, respectively, are shown in Scheme 1. The products were purified by flash chromatography and analyzed by ^{13}C NMR and ^{1}H NMR to identify the esterified position. Based on the general strategy described by Yoshimoto et al.,[24] acylation of a hydroxyl group of substrate will lead to the O-acylated carbon ($^{*}CH_2OCOR$) downfield shift, while the neighboring carbon ($^{*}CCH_2OCOR$) upfield shift in ^{13}C NMR. Characterization of the products **1a** and **2a** revealed that mephenesin and chlorphenesin were acylated at C-1 positions, respectively. The signal for C-1 of mephenesin shifted downfield from 64.1 to 65.7 ppm and that of C-2 shifted upfield from 70.7 to 68.6 ppm. The signal for C-1 of chlorphenesin shifted downfield from 63.8 to 65.5 ppm and that of C-2 shifted upfield from 70.5 to 68.4 ppm. Likewise, analysis of ^{13}C NMR spectra of other products (**1b**–**1d** and **2b**–**2d**) showed that acylations occurred at the primary hydroxyl of mephenesin and chlorphenesin. The analysis of ^{1}H NMR spectra also confirmed the products' structure.

2.2. Effect of enzyme

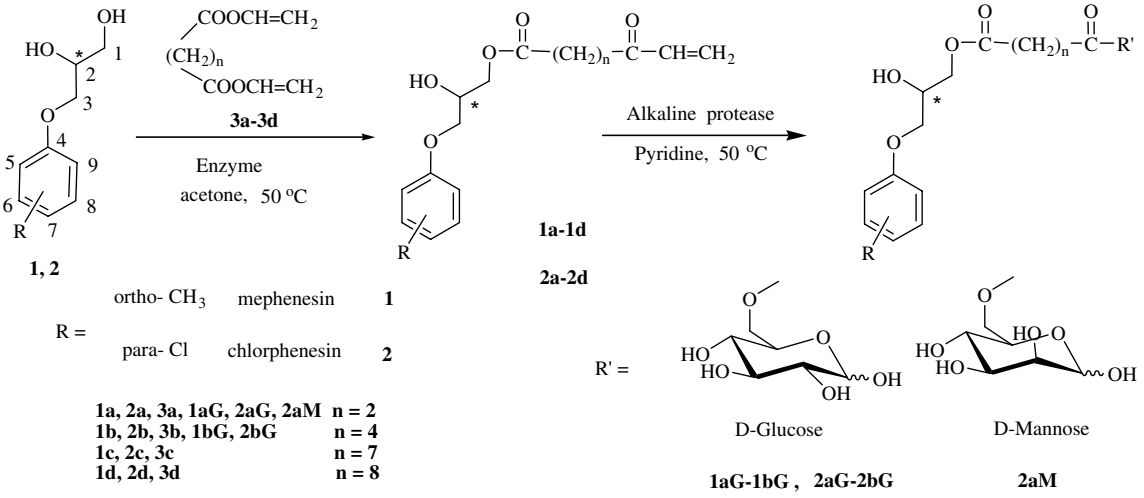
In order to identify suitable enzymes with high transesterification activity in the synthesis of drug vinyl esters, ten commercially available enzymes were tested for the transesterification of mephenesin and chlorphenesin with divinyl adipate in acetone at 50 °C. The screening results are presented and compared in Table 1. The corresponding control experiment in the absence of enzyme formed drug vinyl esters in yields less than 0.5%.

Enzymes derived from various sources such as bacteria, yeast, and molds show different properties, including stability in organic solvent, activity, and specificity. As shown in Table 1, the yield of **1b** catalyzed by the ten enzymes ranged from 0.8% to 78.5%. Most of lipases were appropriate for the transesterification of mephenesin and divinyl adipate except lipase from *C. cylindracea* with a yield of 16.5%. The yield of the **2b** catalyzed by the ten enzymes ranged from 1.9% to 72.5%. Two proteases in this research showed low activity for two drugs.

All investigated enzymes for the transesterification were found to catalyze exclusively the formation of the mono-substituted 1-*O*-vinyladipoyl-substrate in the initial reaction stage. After 24 h, the disubstituted products of two drugs were found in the yields in 0.3–4.3% with all investigated enzymes (the data not shown), and had no obvious increase with extending the reaction time. The best result was obtained from Lipozyme® for chlorphenesin and Candida *antarctica* lipase acrylic resin for mephenesin. Therefore, we selected *C. antarctica* lipase acrylic resin and Lipozyme® for further investigation.

2.3. Effect of organic solvents

Reaction media play a crucial role on maintaining enzyme catalytic activity and stability.[25] To optimize reaction conditions for enzymatic transesterification of mephenesin and chlorphenesin, sixteen different solvents



Scheme 1. High regioselective enzymatic synthesis of drug vinyl esters and amphipathic drug–saccharide derivatives.

Table 1. Effect of enzyme on synthesis of vinyl esters of mephenesin and chlorphenesin

Enzyme	Yield (%)[a]	
	1b	**2b**
Control, no enzyme	<0.5	<0.8
Alkaline protease from *Bacillus subtilis*	0.8	1.9
Amano protease PS from *Aspergillus melleus*	28.7	33.9
Lipozyme® immobilized lipase from *Mucor miehei*	56.0	72.5
Lipase from porcine pancreas	56.4	70.7
Lipase from hog pancreas	57.7	67.6
Lipase from *Candida cylindracea*	16.5	24.8
Lipase Type VII from *Candida rugosa*	65.5	21.8
Lipase AY30	61.1	34.5
Amano Lipase M from *Mucor javanicusr*	34.3	41.3
Candida antarctica lipase acrylic resin	78.5	65.4

Conditions: Enzyme (15 mg mL^{-1}), acetone (2 mL), mephenesin or chlorphenesin (1 mmol), divinyl adipate (6 mmol), 50 °C, 250 rpm, 2 h.
[a] Determined by HPLC.

Table 2. Effect of solvent on synthesis of vinyl esters of mephenesin and chlorphenesin

Entry	Solvent	log *P*	Yield (%)[a]	
			1b	**2b**
1	DMSO	−1.3	63.9	41.1
2	DMF	−1.0	61.2	50.6
3	Dioxane	−0.5	64.5	52.3
4	Acetonitrile	−0.39	65.2	48.4
5	Acetone	−0.23	78.5	72.5
6	Tetrahydrofuran	0.46	58.4	40.2
7	Dichloromethane	0.6	59.0	44.8
8	Pyridine	0.65	66.7	27.8
9	2-Methyl-2-propanol	0.79	58.2	27.1
10	1,2-Dichloroethane	1.2	49.8	46.2
11	Isopropyl ether	1.9	12.5	14.9
12	Toluene	2.2	24.1	10.8
13	Tetrachloromethane	2.9	45.3	22.6
14	Cyclohexane	3.4	5.7	3.2
15	Hexane	3.9	3.8	4.1
16	Octane	4.9	4.4	5.2

Conditions: *Candida antarctica* lipase acrylic resin or Lipozyme® (15 mg mL^{-1}), solvent (2 mL), mephenesin or chlorphenesin (1 mmol), divinyl adipate (6 mmol), 50 °C, 250 rpm, 2 h.
[a] Determined by HPLC.

with log *P* value ranging from −1.3 to 4.9 were screened. There was a certain correlation between the yields and log *P* of solvents.

The solvents with log *P* value ranging from −1.3 to 1.2 were appropriate to the transesterification. The yields of **1b** and **2b** were 49.8–78.5% and 40.2–72.5%, respectively (entries 1–10). The solvents with log *P* greater than 1.2 are unfavorable for the reaction except tetrachloromethane (log *P* 2.9, entry 13), which was due to its good solubility for drugs. The best results were obtained in anhydrous acetone which was a green solvent with lower toxicity and easier processing. Consequently, anhydrous acetone was chosen as reaction medium in the following investigations (Table 2).

2.4. Influence of chain length of acylation agent on initial reaction rates and yields

The influence of the chain length of acylation agent on yields was evaluated. The enzymatic reactivity was found to decrease as the length of the chain of the divinyl dicarboxylates increased. As shown in Figure 1, the equilibrium time of reaction prolonged with the chain length increasing. The yield of **1a** had already been greater than 82% after 30 min, while the yields of **1c**–**1d** were only 65.0% and 61.3% even after prolonged 4 h, respectively, and had no obvious increase with extending the reaction time. The reaction result of **2a**–**2d** was similar to that of mephenesin. Comparing with acylation agents **3b**–**3d**, the reactivity of **3a** was the highest. The initial reaction rate in the transesterification of **3a** with **1** and **2** was high up to 326.4 and 283.8 mM h^{-1}, respectively (Table 3).

2.5. Enzymatic regioselective synthesis of amphipathic drug–saccharide derivatives

Saccharides are hydrophilic compounds and play an important role in living systems. They are particularly effective for the improvement of drugs water-solubility, and dissolution behavior. Our approach is based on a strategy of two-step enzymatic acylation. In the first step, mephenesin and chlorphenesin were reacted with divinyl dicarboxylate to give active acyl derivatives, which were then used as an acyl donor in the second step. Then, we synthesized successfully products **1aG**–**1bG, 2aG**–**2bG**, and **2aM** using alkaline protease from *Bacillus subtilis* as the catalyst, which was confirmed to be an efficient catalyst in the acylation of sugars in our

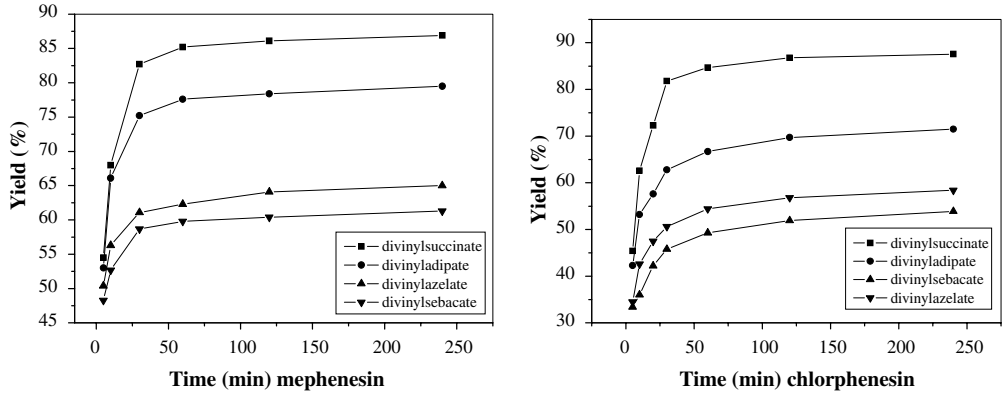


Figure 1. Progress curve of enzymatic acylation of mephenesin and chlorphenesin with different divinyl dicarboxylates.

Table 3. Influence of chain length of acylation agent on the initial reaction rates and yields of the transesterification

Acylation agents	Time (min)	Yields[a](%)		Initial rate (mM h^{-1})	
		Mephenesin	Chlorphenesin	Mephenesin	Chlorphenesin
2a	60	86.2	84.7	326.4	283.8
2b	120	78.5	72.5	260.4	111.0
2c	120	64.1	56.8	87.0	85.8
2d	120	60.4	51.9	84.6	52.8

Conditions: *Candida antarctica* lipase acrylic resin or Lipozyme® (15 mg mL^{-1}), mephenesin or chlorphenesin (1 mmol), divinyl dicarboxylates (6 mmol), acetone (2 mL), 50 °C, 250 rpm.
[a] Determined by HPLC.

previous researches.[26] The position of acylation in enzymatically prepared drug–saccharide derivatives was verified by ^{13}C NMR and ^{1}H NMR. Glucose and mannose were both substituted at C-6 position. The alkaline protease from *B. subtilis* showed an effective regioselectivity in the transesterification of saccharides with vinyl esters of mephenesin and chlorphenesin.

Anomeric mixtures were obtained for D-glucose (α:β = 40:60) in good yields 70.2–78.4% and D-mannose (α:β = 60:40) in yield 64.8%. We monitored the formation of products by TLC. Results indicated that D-glucose reacted faster than D-mannose. No product was detected in the absence of enzyme.

2.6. Lipophilicity of the prodrugs

Lipophilicity is well known as a prime physicochemical descriptor of drugs with relevance to their biological properties. The apparent partition coefficients ($\log P$) of the prodrugs and the parent drugs were investigated in an *n*-octanol/phosphate buffer solution (PBS) of pH 7.4. The concentration of the compounds in *n*-octanol and PBS layer was determined by HPLC. The results are listed in Table 4.

The prodrugs **1a** and **2a** with $\log P$ 2.16 and 2.44 were more lipophilic than their parent drugs **1** and **2**, which would be characterized by enhanced intestinal absorption relative to the parent drug. With the longer carbon chain of vinyl esters, the prodrugs **1b**–**1d** and **2b**–**2d** should have more lipophilicity than **1a** and **2a**.

Compared with the parent drugs, the prodrugs **1aG** and **2aG** had not only the lipid solubility but also the aqueous solubility, which was attributed to modifications of prodrug with carbon chain and glucose functional groups. For chlorphenesin–mannose derivative **2aM**, it was also amphipathic properties. The value of $D_{7.4}$ (distribution coefficient in an *n*-octanol/PBS) was 1.18, which was similar to the value of $D_{7.4}$ **2aG** 1.04. With the increased carbon chain of drug–saccharide derivatives, **1bG** and **2bG** had greater lipophilicity with values of $D_{7.4}$ 3.86 and 4.31, respectively, than **1aG** and **2aG**. The amphipathy of drug–saccharide derivatives was obvious when **1a** and **2a** were used as acylation agents. It was the four carbon chain length of drug vinyl esters that were compatible with the structure of amphipathic drugs.

Table 4. The lipophilic parameters of the prodrugs and the parent drugs

Drug	$\lambda_{detection}$ (nm)	Lipophilicity[a]	$\log P$
1	228	12.2	1.09
2	230	27.5	1.44
1a	228	143.4	2.16
1aG	230	1.10	0.04
1bG	230	3.86	0.59
2a	232	277.1	2.44
2aG	230	1.04	0.02
2bG	232	4.31	0.63
2aM	230	1.18	0.07

Eluent: methanol/water (80:20, v/v).
[a] $D_{7.4}$, distribution coefficient in *n*-octanol/phosphate buffer (pH 7.4).

2.7. Hydrolysis studies in vitro

The kinetics of chemical hydrolysis of **1a** and **2a** as well as its glucose derivatives **1aG** and **2aG** was investigated in simulated gastric fluid (hydrochloric acid buffer, pH 1.2) and simulated phosphate buffer of pH 7.4 at 37 °C. The variation of ester concentration was monitored by HPLC, and the results indicated that the reaction displayed pseudo-first-order kinetics. The rate constants (k_{obsd}) for the individual hydrolysis reaction were calculated from the linear regression equations. The corresponding half-life for the respective prodrugs was then calculated. The results are summarized in Table 5.

The hydrolysis rates of prodrugs **1aG** and **2aG** were slower than those of **1a** and **2a** in two buffer solutions. In hydrochloric acid buffer, the hydrolysis rate of prodrug **1aG** was $5.04 \times 10^{-2}\ h^{-1}$, while that of **1a** was $7.92 \times 10^{-2}\ h^{-1}$. The half-life time of **1aG** was also longer than **1a** in pH 1.2. Compared with **1a** and the parent drug, **1aG** was of sustained release, especially more obvious in PBS. The half-life time of **1aG** in PBS was 6-fold

Table 5. Kinetic data for chemical hydrolysis of the prodrugs

Compound	pH 1.2[a]		pH 7.4[b]	
	k_{obsd} (h^{-1}) × 10^2	$t_{1/2}$ (h)	k_{obsd} (h^{-1}) × 10^2	$t_{1/2}$ (h)
1a	7.92	8.75	3.49	19.86
1aG	5.04	13.75	0.746	92.90
2a	8.07	8.59	3.68	18.83
2aG	5.04	13.75	0.822	84.31

[a] hydrochloric acid buffer (0.2 M).
[b] phosphate buffer (0.2 M).

more than that in pH 1.2. The hydrolysis of chlorphenesin prodrugs was similar to that of mephenesin prodrugs. Presumably, it was the amphipathy of **1aG** and **2aG** that acted as self-stabilizing property due to protection of the hydrolytically labile prodrug linkage especially in PBS, and extension of hydrolysis half-lives in vitro.

3. Conclusion

In conclusion, in order to achieve amphipathic and small molecule prodrugs with saccharide bioactive moieties, we selected mephenesin and chlorphenesin as substrates. A highly regioselective enzymatic synthesis approach was described by the transesterification of mephenesin and chlorphenesin with divinyl dicarboxylates containing different carbon chain length. The influences of enzyme source, organic solvent, reaction time, and chain length of acylation agent on the initial reaction rates and yields were systematically studied. After obtaining eight drug analogues with vinyl groups, we achieved a series of amphipathic drug–saccharide derivatives by sequential regioselective acylation with D-glucose and D-mannose. Through investigation of the lipophilicity and hydrolysis in vitro, drug–saccharide derivatives showed amphipathy and obvious sustained release characteristics compared with parent drugs. The strategy was efficient and facile for synthesis of amphipathic small molecule prodrugs with saccharide. The further research of other relevant amphipathic prodrug derivatives is in progress.

4. Experimental

4.1. Materials

Lipozyme® (EC 3.1.1.1, an immobilized preparation of lipase from *Mucor miehei*, 42 U/g), lipase from porcine pancreas (EC 3.1.1.3, Type II, powder, 30–90 U/mg), lipase from *C. cylindracea* (EC 3.1.1.3, powder, 2.8 U/mg), and lipase from hog pancreas (EC 3.1.1.3, powder, 2.4 U/mg) were purchased from Fluka. *C. antarctica* lipase acrylic resin (EC 3.1.1.3, an immobilized preparation of lipase from *C. antarctica* on macroporous acrylic resin, 10,000 U/g,) and lipase Type VII from *Candida rugosa* (EC 3.1.1.3, powder, 706 U/mg) were purchased from Sigma. Amano Lipase M from *Mucor javanicus* (EC 3.1.1.3, powder, 10 U/mg) and Amano Protease PS from *Aspergillus melleus* (EC 3.4.21.63, powder, 100 U/mg) were purchased from Aldrich. Lipase AY30 (EC 3.1.1.3, powder) was purchased from Acrös. Alkaline protease from *B. subtilis* (EC 3.4.21.14, a crude preparation of the alkaline serine protease, 100 U/mg) was purchased from Wuxi Enzyme Co. Ltd (Wuxi, PR China). Mephenesin and chlorphenesin were purchased from Alfa Aesar, a Johnson Matthey Company. All the enzymes were used directly in commercial preparations without further purification. All solvents were of analytical grade and were dried by storing over activated 3 Å molecular sieves before use. All other reagents were used as received.

4.2. Analytical methods

The process of reactions was monitored by TLC on silica with petroleum ether/EtOAc (2:1, v/v) as solvent for the monoester compounds (**1a–1d, 2a–2d**) and EtOAc/methanol/water (17:2:1, v/v) for the drug–saccharide derivatives (**1aG–1bG, 2aG–2bG, 2aM**). The ^{1}H NMR and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm) and a UV detector (275 nm). Methanol/water (80:20, v/v) was used as a mobile phase, while the flow rate was adjusted to 1 mL min^{-1}.

4.3. General procedure for synthesis of drug vinyl esters

The reaction was initiated by adding 15 mg/mL Lipozyme® to 20 mL acetone containing 5 mmol drugs and 30 mmol divinyl dicarboxylates (**3a**–**3d**), respectively. The suspension was kept at 50 °C and shaken at 250 rpm. The reaction was monitored by TLC (petroleum ether/ethyl acetate 3:2, v/v). The reactions were terminated by filtering off the enzyme and then the filtrate was evaporated under reduced pressure. The products were isolated by silica gel column chromatography with an eluent consisting of petroleum ether/ethyl acetate (2:1, v/v). Enzymatic synthesis of vinyl esters of mephenesin and chlorphenesin is shown in Scheme 1.

4.4. Synthesis of 1-*O*-vinylsuccinyl-mephenesin (1a)

The reaction time was 1 h and the product yield was 85.2%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.25 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH═), 7.15 (t, 2H, Ar–H), 6.88 (d, 1H, Ar–H), 6.81 (d, 1H, Ar–H), 4.90 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, ═CH_2), 4.58 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, ═CH_2), 4.38–4.26 (m, 3H, –C*H*(OH)CH_2–), 4.02 (d, 2H, J = 5.0 Hz, –OCH_2CH–), 2.72 (m, 4H, –CH_2CH_2–), 2.58 (br, 1H, –CH(O*H*)–), 2.22 (s, 3H, –CH_3). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.7, 171.0 (C═O), 156.5, 131.0, 127.1, 126.9, 121.2, 111.1 (Ar, mephenesin), 141.4 (–O–CH═), 98.1 (═CH_2), 68.7 (C-3, mephenesin), 68.6 (C-2, mephenesin), 65.7 (C-1, mephenesin), 29.2, 29.1 (–CH_2–), 16.3 (C-4, mephenesin). IR (KBr, cm^{-1}): 3495 (OH), 1750, 1742 (O–C═O), 3091, 1647 (C═C), 1603, 1591, 1496, 753, 714 (Ar). ESI-MS (*m*/*z*): 331 [M+Na]$^{+}$.

4.5. Synthesis of 1-*O*-vinyladipoyl-mephenesin (1b)

The reaction time was 2 h and the product yield was 78.5%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.25 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH═), 7.15 (t, 2H, Ar–H), 6.88 (d, 1H, Ar–H), 6.81 (d, 1H, Ar–H), 4.87 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, ═CH_2), 4.56 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, ═CH_2), 4.35–4.26 (m, 3H, –C*H*(OH)CH_2–), 4.02 (d, 2H, J = 5.0 Hz, –OCH_2CH–),

3.24 (br, 1H, –CH(O*H*)–), 2.39 (m, 4H, 2-CH_2), 2.22 (s, 3H, –CH_3), 1.68 (m, 4H, 2-CH_2). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.5, 170.6 (C=O), 156.5, 130.9, 127.0, 126.9, 121.1, 111.2, (Ar, mephenesin), 141.4 (–O–CH=), 98.0 (=CH_2), 68.7 (C-3, mephenesin), 68.6 (C-2, mephenesin), 65.7 (C-1, mephenesin), 33.8, 33.5, 24.3, 24.0 (–CH_2–), 16.3 (C-4, mephenesin). IR (KBr, cm^{-1}): 3498 (OH), 1749, 1738 (O–C=O), 3092, 1647 (C=C), 1597, 1591, 1493, 750, 719 (Ar). ESI-MS (*m*/*z*): 359 $[M+Na]^+$.

4.6. Synthesis of 1-*O*-vinylazeloyl-mephenesin (1c)

The reaction time was 2 h and the product yield was 64.1%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.28 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.15 (t, 2H, Ar–H), 6.89 (d, 1H, Ar–H), 6.81 (d, 1H, Ar–H), 4.87 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.56 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.35–4.26 (m, 3H, –C*H*(OH)CH_2–), 4.02 (d, 2H, J = 5.0 Hz, –OCH_2CH–), 2.68 (br, 1H, –CH(O*H*)–), 2.36 (m, 4H, 2-CH_2), 2.23 (s, 3H, –CH_3), 1.63 (m, 4H, 2-CH_2), 1.31 (m, 6H, 3-CH_2). ^{13}C NMR ($CDCl_3$, δ, ppm): 174.2, 170.8 (C=O), 156.5, 131.0, 127.0, 127.0, 121.1, 111.3, (Ar, mephenesin), 141.4 (–O–CH=), 98.0 (=CH_2), 68.7 (C-3, mephenesin), 68.6 (C-2, mephenesin), 65.7 (C-1, mephenesin), 34.3, 34.0, 29.0, 29.0, 29.0, 25.0, 24.7 (–CH_2–), 16.3 (C-4, mephenesin). IR (KBr, cm^{-1}): 3495 (OH), 1748, 1740 (O–C=O), 3092, 1647 (C=C), 1603, 1591, 1496, 753, 714 (Ar). ESI-MS (*m*/*z*): 401 $[M+Na]^+$.

4.7. Synthesis of 1-*O*-vinylsebacoyl-mephenesin (1d)

The reaction time was 2 h and the product yield was 60.0%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.28 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.15 (t, 2H, Ar–H), 6.89 (d, 1H, Ar–H), 6.80 (d, 1H, Ar–H), 4.87 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.56 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.35–4.26 (m, 3H, –C*H*(OH)CH_2–), 4.02 (d, 2H, J = 5.0 Hz, –OCH_2CH–), 2.68 (br, 1H, –CH(O*H*)–), 2.36 (m, 4H, 2-CH_2), 2.23 (s, 3H, –CH_3), 1.63 (m, 4H, 2-CH_2), 1.28 (m, 8H, 4-CH_2). ^{13}C NMR ($CDCl_3$, δ, ppm): 174.2, 171.1 (C=O), 156.5, 131.0, 127.0, 127.0, 121.2, 111.2, (Ar, mephenesin), 141.3 (–O–CH=), 98.1 (=CH_2), 68.7 (C-3, mephenesin), 68.6 (C-2, mephenesin), 65.7 (C-1, mephenesin), 34.3, 34.1, 29.2, 29.2, 29.1, 29.1, 25.0, 24.7 (–CH_2–), 16.4 (C-4, mephenesin). IR (KBr, cm^{-1}): 3495 (OH), 1750, 1742 (O–C=O), 3093, 1647 (C=C), 1603, 1591, 1496, 753, 714 (Ar). ESI-MS (*m*/*z*): 415 $[M+Na]^+$.

4.8. Synthesis of 1-*O*-vinylsuccinyl-chlorphenesin (2a)

The reaction time was 1 h and the product yield was 84.7%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.26 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.23 (d, 2H, Ar–H), 6.85 (d, 2H, Ar–H), 4.91 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.58 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.35-4.24 (m, 3H, –C*H*(OH)CH_2–), 4.00 (m, 2H, –OCH_2CH–), 2.74 (m, 4H, –CH_2CH_2–). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.7, 171.0 (C=O), 157.2, 129.6, 129.6, 126.3, 116.0, 116.0 (Ar, chlorphenesin), 141.4 (–O–CH=), 98.1 (=CH_2), 69.2 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.5 (C-1, chlorphenesin), 29.2, 29.1 (–CH_2–). IR (KBr, cm^{-1}): 3528 (OH), 1762, 1740 (O–C=O), 1647 (C=C), 1597, 1499, 836, 806 (Ar). (ESI-MS (*m*/*z*): 351 $[M+Na]^+$.

4.9. Synthesis of 1-*O*-vinyladipoyl-chlorphenesin (2b)

The reaction time was 2 h and the product yield was 72.5%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.27 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.57 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.32–4.21 (m, 3H, –C*H*(OH)CH_2–), 3.98 (m, 2H, –OCH_2CH–), 3.05 (br, 1H, –CH(O*H*)–), 2.39 (m, 4H, 2-CH_2–), 1.68 (m, 4H, 2-CH_2–). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.5, 170.6 (C=O), 157.1, 129.5, 129.5, 126.3, 116.0, 116.0 (Ar, chlorphenesin), 141.2 (–O–CH=), 98.0 (=CH_2), 69.2 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.4 (C-1, chlorphenesin), 33.8, 33.5, 24.3, 24.0 (–CH_2–). IR (KBr, cm^{-1}): 3515 (OH), 1760, 1736 (O–C=O), 1647 (C=C), 1592, 1497, 836, 806 (Ar). ESI-MS (*m*/*z*): 379 $[M+Na]^+$.

4.10. Synthesis of 1-*O*-vinylazeloyl-chlorphenesin (2c)

The reaction time was 2 h and the product yield was 56.8%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.27 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.57 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.32–4.21 (m, 3H, –C*H*(OH)CH_2–), 3.99 (m, 2H, –OCH_2CH–), 3.60 (br, 1H, –CH(O*H*)–), 2.36 (m, 4H, 2-CH_2–), 1.64 (m, 4H, 2-CH_2–), 1.32 (m, 6H, 3-CH_2–). ^{13}C NMR ($CDCl_3$, δ, ppm): 174.2, 170.8 (C=O), 157.1, 129.7, 129.6, 126.4, 116.0, 116.0 (Ar, chlorphenesin), 141.2 (–O–CH=), 98.0 (=CH_2), 69.3 (C-3, chlorphenesin), 68.4 (C-2, chlorphenesin), 65.3 (C-1, chlorphenesin), 34.3, 34.0, 29.0, 29.0, 29.0, 25.0, 24.7 (–CH_2–). IR (KBr, cm^{-1}): 3520 (OH), 1757, 1734 (O–C=O), 1645 (C=C), 1592, 1497, 836, 806 (Ar). ESI-MS (*m*/*z*): 421 $[M+Na]^+$.

4.11. Synthesis of 1-*O*-vinylsebacoyl-chlorphenesin (2d)

The reaction time was 2 h and the product yield was 51.9%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.27 (dd, 1H, J = 6.3 Hz, J = 14.1 Hz, –CH=), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, J = 14.1 Hz, J = 6.3 Hz, =CH_2), 4.57 (dd, 1H, J = 6.3 Hz, J = 1.5 Hz, =CH_2), 4.30–4.22 (m, 3H, –C*H*(OH)CH_2–), 3.99 (m, 2H, –OCH_2CH–), 2.80 (br, 1H, –CH(O*H*)–), 2.36 (m, 4H, 2-CH_2–), 1.63 (m, 4H, 2-CH_2–), 1.27 (m, 8H, 4-CH_2–). ^{13}C NMR ($CDCl_3$, δ, ppm): 174.2, 171.18 (C=O), 157.2, 129.5, 129.5, 126.4, 116.0, 116.0 (Ar, chlorphenesin), 141.3 (–O–CH=), 98.1 (=CH_2), 69.3 (C-3, chlorphenesin), 68.5 (C-2, chlorphenesin), 65.4 (C-1, chlorphenesin), 34.3, 34.1, 29.2, 29.2, 29.1, 29.1, 25.0, 24.7 (–CH_2–). IR (KBr, cm^{-1}): 3519 (OH), 1757, 1734 (O–C=O), 1642 (C=C), 1592, 1497, 832, 803 (Ar). ESI-MS (*m*/*z*): 435 $[M+Na]^+$.

4.12. General procedure for synthesis of amphipathic drug–saccharide derivatives

A mixture of **1a**–**1b** or **2a**–**2b** (2 mmol) and D-glucose (4 mmol) or D-mannose, alkaline protease from *B. subtilis* (0.25 g, 25 mg/mL), and 10 mL pyridine was shaken at 250 rpm for 36 h at 50 °C. The reactions were terminated by filtering off the enzyme. The pyridine was evaporated. Formations of the drug–saccharide derivatives were confirmed by TLC. The product was isolated by silica gel chromatography with an eluent consisting of ethyl acetate/methanol/water (17:2:1, by vol) to give products (**1aG**–**1bG**, **2aG**–**2bG**, **2aM**). Regioselective enzymatic synthesis of drug–saccharide derivatives is shown in Scheme 1.

4.13. Synthesis of 6-*O*-(1-*O*-mephenesin-vinylsuccinyl)-D-glucose (1aG)

The isolated yield of product was 77.7%. ^{1}H NMR (D_2O, δ, ppm): 6.88 (d, 1H, Ar–H), 6.78 (d, 1H, Ar–H), 6.79 (t, 2H, Ar–H), 5.12 (d, 0.40H, J = 3.60, H-1 of α-D-glucose), 4.58 (d, 0.60H, J = 7.91, H-1 of β-D-glucose), 4.35–4.12 (m, 2H, H-6, 6′ of β-D-glucose, H-6, 6′ of α-D-glucose), 4.03–3.18 (m, 9H,–OCH_2CHCH_2–, α H or β H of D-glucose), 2.53 (m, 4H, 2-CH_2–), 1.96 (s, 3H, -CH_3). ^{13}C NMR (D_2O, δ, ppm): 174.2, 174.1 (C=O), 156.5, 130.8, 127.1, 126.8, 121.0, 111.7 (C–Ar, mephenesin), 96.4 (C1 of β-D-glucose), 92.4 (C1 of α-D-glucose), 75.9 (C3 of β-D-glucose), 74.4 (C2 of β-D-glucose), 73.7 (C5 of β-D-glucose), 73.0 (C3 of α-D-glucose), 71.8 (C2 of α-D-glucose), 70.1 (C4 of α-D-glucose), 70.0 (C4 of β-D-glucose), 69.4 (C5 of α-D-glucose), 68.6 (C-3, mephenesin), 67.9 (C-2, mephenesin), 66.0 (C-1, mephenesin), 63.7 (C6 a, β of D-glucose), 29.2, 29.1 (–CH_2–), 16.3 (C-4, mephenesin). IR (cm^{-1}): 3340 (OH), 2960, 2850 (CH), 1735 (C=O), 1468 (CH_2), 1149 (C–O), 1058 (C–O–H). ESI-MS (*m*/*z*): 467 $[M+Na]^+$.

4.14. Synthesis of 6-*O*-(1-*O*-mephenesin-vinyladipoyl)-D-glucose (1bG)

The isolated yield of product was 73.6%. ^{1}H NMR (D_2O, δ, ppm): 6.88 (d, 1H, Ar–H), 6.78 (d, 1H, Ar–H), 6.79 (t, 2H, Ar–H), 5.12 (d, 0.40H, J = 3.60, H-1 of α-D-glucose), 4.58 (d, 0.60H, J = 7.91, H-1 of β-D-glucose), 4.35–4.12 (m, 2H, H-6, 6′ of β-D-glucose, H-6, 6′ of α-D-glucose), 4.03–3.18 (m, 9H,–OCH_2CHCH_2–, α H or β H of D-glucose), 2.37 (m, 4H, 2-CH_2), 1.98 (s, 3H, –CH_3), 1.66 (m, 4H, 2-CH_2). ^{13}C NMR (D_2O, δ, ppm): 174.2, 174.1 (C=O), 156.5, 130.8, 127.1, 126.8, 121.0, 111.7 (C–Ar, mephenesin), 96.4 (C1 of β-D-glucose), 92.4 (C1 of α-D-glucose), 75.9 (C3 of β-D-glucose), 74.4 (C2 of β-D-glucose), 73.7 (C5 of β-D-glucose), 73.0 (C3 of α-D-glucose), 71.8 (C2 of α-D-glucose), 70.1 (C4 of α-D-glucose), 70.0 (C4 of β-D-glucose), 69.4 (C5 of α-D-glucose), 68.6 (C-3, mephenesin), 67.9 (C-2, mephenesin), 66.0 (C-1, mephenesin), 63.7 (C6 a, β of D-glucose), 33.8, 33.5, 24.3, 24.0 (–CH_2–), 16.3 (C-4, mephenesin). IR (cm^{-1}): 3338 (O–H), 2963, 2850 (C–H), 1734 (C=O), 1468 (CH_2), 1149 (C–O), 1058 (C–O–H). ESI-MS (*m*/*z*): 495 $[M+Na]^+$.

4.15. Synthesis of 6-*O*-(1-*O*-chlorphenesin-vinylsuccinyl)-D-glucose (2aG)

The isolated yield of product was 78. 4%. ^{1}H NMR (D_2O, δ, ppm): 6.88 (d, 2H, Ar–H), 6.56 (d, 2H, Ar–H), 5.12 (d, 0.40H, J = 3.60, H-1 of α-D-glucose), 4.55 (d, 0.60H, J = 7.91, H-1 of β-D-glucose), 4.31–4.12 (m, 2H, H-6, 6′ of β-D-glucose, H-6, 6′ of α-D-glucose), 4.03-3.18 (m, 9H, –OCH_2CHCH_2–,α H or β H of D-glucose), 2.52 (m, 4H, 2-CH_2–). ^{13}C NMR (D_2O, δ, ppm): 174.2, 174.1 (C=O), 157.1, 129.5, 129.5, 126.0, 116.2, 116.2 (C–Ar, chlorphenesin), 96.4 (C1 of β-D-glucose), 92. 5 (C1 of α-D-glucose), 76.1 (C3 of β-D-glucose), 74.5 (C2 of β-D-glucose), 73.7 (C5 of β-D-glucose), 73.0 (C3 of α-D-glucose), 71.8 (C2 of α-D-glucose), 70.1 (C4 of α-D-glucose), 70.0 (C4 of β-D-glucose), 69.9 (C5 of α-D-glucose), 69.5 (C-3, chlorphenesin), 67.8 (C-2, chlorphenesin), 65.8 (C-1, chlorphenesin), 63.7 (C6 a, β of D-glucose), 29.2, 29.0 (–CH_2–). IR (cm^{-1}): 3364 (O–H), 1739 (C=O). ESI-MS (*m*/*z*): 487 $[M+Na]^+$.

4.16. Synthesis of 6-*O*-(1-*O*-chlorphenesin-vinyladipoyl)-D-glucose (2bG)

The isolated yield of product was 70. 2%. ^{1}H NMR (D_2O, δ, ppm): 6.88 (d, 2H, Ar–H), 6.56 (d, 2H, Ar–H), 5.12 (d, 0.40H, J = 3.60, H-1 of α-D-glucose), 4.55 (d, 0.60H, J = 7.91, H-1 of β-D-glucose), 4.31–4.12 (m, 2H, H-6, 6′ of β-D-glucose, H-6, 6′ of α-D-glucose), 4.03-3.18 (m, 9H, –OCH_2CHCH_2–,α H or β H of D-glucose), 2.52 (m, 4H, 2-CH_2–). ^{13}C NMR (D_2O, δ, ppm): 174.2, 174.1 (C=O), 157.1, 129.5, 129.5, 126.0, 116.2, 116.2 (C–Ar, chlorphenesin), 96.4 (C1 of β-D-glucose), 92. 5 (C1 of α-D-glucose), 76.1 (C3 of β-D-glucose), 74.5 (C2 of β-D-glucose), 73.7 (C5 of β-D-glucose), 73.0 (C3 of α-D-glucose), 71.8 (C2 of α-D-glucose), 70.1 (C4 of α-D-glucose), 70.0 (C4 of β-D-glucose), 69.9 (C5 of α-D-glucose), 69.5 (C-3, chlorphenesin), 67.8 (C-2, chlorphenesin), 65.8 (C-1, chlorphenesin), 63.7 (C6 a, β of D-glucose), 29.2, 29.0 (–CH_2–). IR (cm^{-1}): 3364 (O–H), 1739 (C=O). ESI-MS (*m*/*z*): 515 $[M+Na]^+$.

4.17. Synthesis of 6-*O*-(1-*O*-chlorphenesin-vinylsuccinyl)-D-mannose (2aM)

6-*O*-(1-*O*-Chlorphenesin-vinylsuccinyl)-D-mannose was synthesized by the same method as for 6-*O*-(1-*O*-chlorphenesin-vinylsuccinyl)-D-glucose. The isolated yield of product was 64. 8%. ^{1}H NMR (D_2O, δ, ppm): 6.98 (d, 2H, Ar–H), 6.62 (d, 2H, Ar–H), 5.07 (s, 0.6H, H-1 of α-D-mannose), 4.76 (s, 0.4H, H-1 of β-D-mannose), 4.31–4.18 (m, 2H, H-6, 6′ of β-D-mannose, H-6, 6′ of α-D-mannose), 4.10-4.04 (m, 3H, –CH(OH)CH_2–), 3.88 (m, 2H, –OCH_2CH–), 3.77–3.51 (m, 4H, α H or β H of D-mannose), 2.56 (m, 4H, 2-CH_2–). ^{13}C NMR (D_2O, δ, ppm): 174.4, 174.3 (C=O), 157.0, 129.5, 129.5, 125.8, 116.2, 116.1 (C–Ar, chlorphenesin), 94.4 (C1 of α-D-mannose), 94.0 (C1 of β-D-mannose), 73.9 (C5 of β-D-mannose), 73.1 (C3 of β-D-mannose), 71.3 (C2 of β-D-mannose), 70.8 (C2 of α-D-mannose), 70.4

(C3 of α-D-mannose), 70.3 (C5 of α-D-mannose), 68.9 (C-3, chlorphenesin), 67.6 (C-2, chlorphenesin), 67.1 (C-1, chlorphenesin), 66.9 (C4 of α-D-mannose), 66.8 (C4 of β-D-mannose), 64.3 (C6 a, *β* of D-mannose), 28.9, 28.9 (-CH2-). IR (cm^{-1}): 3360 (O–H), 1736 (C═O). ESI-MS (*m*/*z*): 487 $[M+Na]^+$.

4.18. Determination of distribution coefficients

The distribution coefficient (*D*) of the prodrugs and the parent drugs was determined by dissolving 5 mg of the respective compound in 2.5 mL of *n*-octanol and adding to an equal volume of phosphate buffer (pH 7.4) in screw-capped test tube. The solutions were then mixed for 15 min and centrifuged at 1×10^4 rpm for 5 min. The layers were separated and aliquots of 50 μL were diluted to 500 μL. Samples of 20 μL were taken and analyzed by HPLC. All experiments were conducted in triplicate and the mean values were taken. The values of $D_{7.4}$ for the respective compounds were then calculated and the results are listed in Table 4.

4.19. Hydrolysis in aqueous solutions in vitro

A 0.2 M hydrochloric acid buffer of pH 1.2 as non-enzymatic simulated gastric fluid (SGF) and a 0.2 M phosphate buffer of pH 7.4 were used in this study. Stock solution of the respective ester prodrug (25 mM) was prepared in dioxane. An aliquot (1 mL) was added to 9 mL of the appropriate thermostated (37 °C) aqueous buffer solution (pH 7.4) to initiate the hydrolysis reaction. Samples of 20 μL were taken at fixed intervals and diluted to 200 μL with methanol, then analyzed by HPLC. Pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of the ester prodrugs were then calculated from the slopes of the lineate plots of log (% residual prodrug) versus time.

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