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Design, Synthesis, and Evaluation of Novel Cyclic Phosphates of 5-Aminosalicylic Acid as Cytochrome P450-Activated Prodrugs

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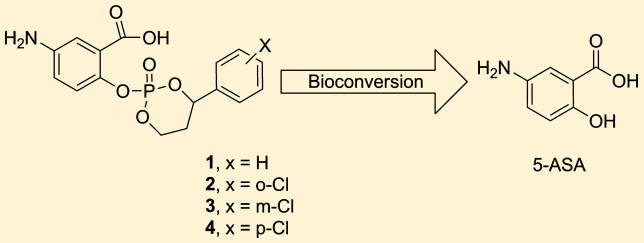
ABSTRACT: Four novel cyclic phosphates of the anti-inflammatory agent 5-aminosalicylic acid (5-ASA) were designed and synthesized as cytochrome P450 (CYP)-activated prodrugs. These prodrugs can be used for targeting into gut wall, since these types of cyclic phosphates are known to be activated mainly by CYP3A forms, which are expressed not only in the liver but also in the small intestine and to a lesser extent in the colon. The present study shows that aromatic ring activating substituents, like chlorine, are definitely needed to obtain the desired enzymatic cleavage of the cyclic phosphate prodrugs of 5-ASA. However, the position of the activating substituent has also a strong impact on the chemical stability, and therefore, an appropriate balance between the rates of prodrug bioactivation and chemical stability needs to be taken into consideration in future studies on cyclic phosphate prodrugs of 5-ASA.



1, x = H
2, x = o-Cl
3, x = m-Cl
4, x = p-Cl

KEYWORDS: *5-aminosalicylic acid, bioactivation/bioconversion, cyclic phosphate, cytochrome P450 enzyme, prodrug*

INTRODUCTION

5-Aminosalicylic acid (5-ASA, mesalamine) is an anti-inflammatory agent primarily used to treat inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease.[1] Local concentrations of 5-ASA in the intestinal mucosa are known to determine the clinical efficacy of 5-ASA. However, when administrated orally, 5-ASA is rapidly and extensively absorbed from the upper gastrointestinal tract, causing unwanted systemic side effects.[2] Several approaches, such as prodrugs and prolonged-released formulations, have been developed to deliver 5-ASA locally to the site of inflammation, i.e., to the distal ileum and/or colon.[3] Classic examples are azo-prodrugs of 5-ASA, such as sulfasalazine, that are activated by colonic bacterial azoreductases. However, all these oral 5-ASA derivatives cause multiple adverse effects. Other prodrug strategies, such as amino acid esters,[4] acrylic-type polymeric prodrugs,[5] conjugates with dendrimer,[6] dextran,[7] and cyclodextrin,[8] as well as liposomal formulations[9] of 5-ASA have also been studied as colon-specific prodrugs with variable results. Being a valuable and widely used agent, more selective methods are needed to improve the targeting of 5-ASA to its site of action.[1]

We have recently reported that cyclic phosphates could serve as potential cytochrome P450 (CYP) 3A4-selective prodrugs of drug molecules having a free hydroxyl group.[10] To date, this prodrug approach has been successfully applied to 18β-glycyrrhetinic acid to gain antiulcer and anti-inflammatory sustained-release effects.[11] Bioconversion of cyclic phosphate prodrugs have been designed to undergo an initial CYP-catalyzed oxidation at the C4 methine primarily by CYP3A forms (Figure 1). The hydroxylated intermediate of the cyclic phosphate is then converted to a free phosphate by β-elimination of aryl vinyl ketone. This highly electrophilic byproduct has been associated with cytotoxicity and genotoxicity. However, it is rapidly and quantitatively detoxified by intracellular glutathione, as long as the glutathione levels remain at 20% of normal liver/gut levels.[12] The liberated free phosphate is subsequently hydrolyzed by phosphatases releasing the parent drug with a free hydroxyl group. In the present study four cyclic phosphates of 5-ASA were designed and synthesized to investigate whether these compounds could serve as CYP-selective prodrugs of 5-ASA. Taking several structure-related requirements into consideration, these types of cyclic phosphate prodrugs can undergo a CYP-catalyzed prodrug cleavage and could be ideal candidates for further studies to target 5-ASA especially to the small intestinal wall.

EXPERIMENTAL SECTION

General Synthetic Methods. All the reactions were performed with reagents of commercial high purity quality without further purification. Reactions were monitored by thin-layer chromatography using aluminum sheets coated with silica gel 60 F_{245} (0.24 mm) with suitable visualization. Purifications by flash chromatography were performed on silica gel 60 (0.063−0.200 mm mesh). ^{1}H, ^{13}C, and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrometer (Bruker Biospin, Fällanden, Switzerland) operating at 500.13, 125.75, and 202.46 MHz, respectively. Tetramethylsilane was used as an internal standard for ^{1}H and ^{13}C spectra and 85% H_3PO_4 as an external standard for ^{31}P

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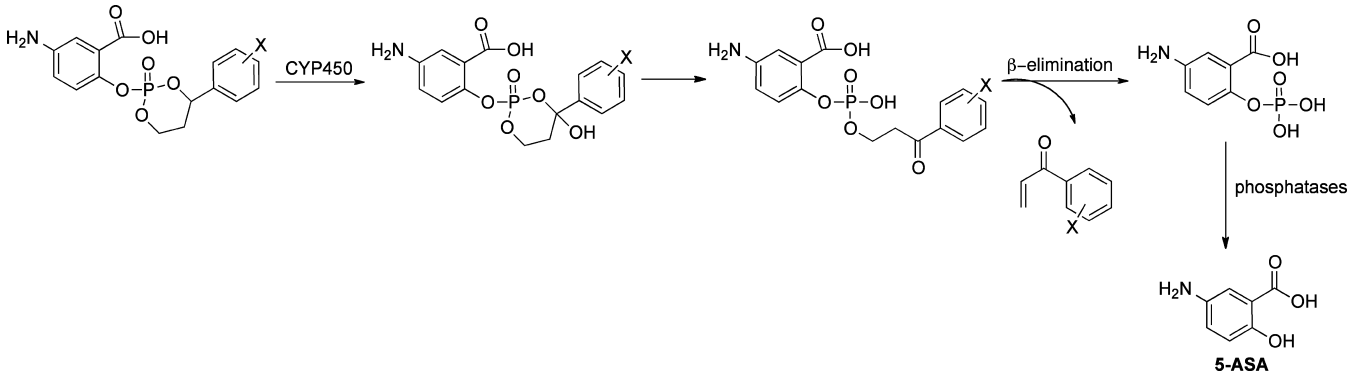

Figure 1. Proposed bioconversion of cyclic phosphate prodrugs to 5-ASA.

spectra. Furthermore, the products were characterized by elemental analysis (C, H, N) with a ThermoQuest CE Instruments EA 1110-CHNS-O elemental analyzer (CE Instruments, Milan, Italy).

General Procedure for the Synthesis of Cyclic Phosphates (1−4). The amine group of 5-ASA was protected by stirring 5-ASA (1 equiv) with di-*tert*-butyl-dicarbonate (1 equiv) and $NaHCO_3$ (1 equiv) in anhydrous DMF at +50 °C overnight, and the residue was purified by flash chromatography eluting with 1−5% MeOH in DCM solution.[13] A solution of the compound **5** (1 equiv) in anhydrous DMF (5 mL) was treated with 1 M *tert*-BuMgCl in THF (1.3 equiv) and stirred at room temperature under argon for 30 min. A selected 4-aryl-2-(4-nitrophenoxy)-2-oxo-1,3,2- dioxaphosphorinane **6**−**9** (1.3 equiv), prepared as previously described,[14−18] in anhydrous THF (2−5 mL) was added to the reaction mixture and stirred overnight at room temperature. The mixture was quenched with saturated NH_4Cl (10 mL) and extracted with EtOAc (20 mL). The organic phase was washed with 1 M NaOH (2 × 20 mL), water (2 × 20 mL), and brine (2 × 20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The *t*-Boc protecting group was removed by stirring the obtained compounds in 3 M HCl solution at room temperature for 1−2 h. The cyclic phosphates **1**−**4** were purified by flash chromatography eluting with 1−5% MeOH in DCM solution.

5-Amino-2-((2-oxido-4-phenyl-1,3,2-dioxaphosphinan-2-yl)oxy)benzoic Acid (1). ^{1}H NMR ($CDCl_3$): δ 2.17−2.35 (2H, m), 4.09−4.27 (2H, m), 4.87−4.91 (1H, m), 7.12−7.32 (6H, m), 8.04−8.08 (2H, m). ^{13}C NMR: δ 39.99, 59.04, 65.58, 120.50, 120.54, 125.63 (2C), 126.81, 128.64, 128.82 (2C), 140.48, 144.57, 155.49, 161.92, 171.04. ^{31}P NMR: δ −6.33 and −6.01 (84:16). Anal. Calcd for ($C_{16}H_{16}NO_6P{\cdot}1.2H_2O{\cdot}0.2DCM$): C, 50.16; H, 4.83; N, 3.61. Found: C, 49.92; H, 4.68; N, 3.59.

5-Amino-2-((4-(2-chlorophenyl)-2-oxido-1,3,2-dioxaphosphinan-2-yl)oxy)benzoic Acid (2). ^{1}H NMR ($CDCl_3$): δ 2.21−2.35 (2H, m), 4.46−4.74 (2H, m), 5.87−6.04 (1H, m), 7.28−7.62 (5H, m), 8.12−8.23 (2H, m). ^{13}C NMR: δ 32.35, 69.02, 79.25, 120.31, 125.74, 125.74, 125.85, 126.93, 127.78, 129.59, 130.11, 131.06, 135.61, 144.78, 155.07, 171.33. ^{31}P NMR: δ −13.69 and −11.26 (81:19). Anal. Calcd for ($C_{16}H_{15}NO_6PCl$ 0.3 H_2O): C, 49.39; H, 4.04; N, 3.60. Found: C, 49.06; H, 4.01; N, 3.42.

5-Amino-2-((4-(3-chlorophenyl)-2-oxido-1,3,2-dioxaphosphinan-2-yl)oxy)benzoic Acid (3). ^{1}H NMR ($CDCl_3$): δ 1.94−2.26 (2H, m), 3.35−4.09 (2H, m), 4.86−5.31 (1H, m), 7.02−7.29 (5H, m), 7.93 (1H, d, $^3J_{HH}$ = 8.1 Hz), 8.07 (1H, d, $^3J_{HH}$ = 7.8 Hz). ^{13}C NMR: δ 29.63, 58.50, 75.62, 119.62, 119.92, 124.97, 125.02, 125.37, 126.99, 128.54, 129.96, 134.41, 142.96, 143.16, 158.06, 171.45. ^{31}P NMR: δ −5.88 and −5.36 (50:50). Anal. Calcd for ($C_{16}H_{15}NO_6PCl{\cdot}1.2DCM$): C, 42.54; H, 3.36; N, 2.88. Found: C, 42.87; H, 3.39; N, 3.28.

5-Amino-2-((4-(4-chlorophenyl)-2-oxido-1,3,2-dioxaphosphinan-2-yl)oxy)benzoic Acid (4). ^{1}H NMR ($CDCl_3$): δ 2.07−2.49 (2H, m), 4.52−4.73 (2H, m), 5.55−5.80 (1H, m), 7.28−7.46 (6H, m), 8.25−8.26 (1H, m). ^{13}C NMR: δ 31.44, 50.76, 81.88, 120.14, 120.19, 120.82, 125.71, 125.91, 126.86, 127.00, 129.14, 135.03, 136.39, 162.59, 163.00, 171.59. ^{31}P NMR: δ −34.73 and −32.34 (65:35). Anal. Calcd for ($C_{16}H_{15}NO_6PCl{\cdot}0.4H_2O{\cdot}0.2DMF$): C, 49.16; H, 4.28; N, 4.14. Found: C, 48.93; H, 3.89; N, 4.12.

High-Performance Liquid Chromatography (HPLC) Analyses. The analyses were performed on the high-performance liquid chromatography (HPLC) system, which consisted of an Agilent 1100 binary pump (Agilent Technologies Inc., Wilmington, DE, USA), a 1100 micro vacuum degasser, an 1100 autosampler, a 1200 variable wavelength detector SL (λ = 200 nm), an 1100 fluorescence detector (λ_{exc} = 310 nm, λ_{emiss} = 500 nm), and an Agilent Zorbax ODS analytical column (4.6 mm × 250 mm, 5 μm). The chromatographic separations for samples from biological matrixes were achieved by using an isocratic elution of 50 mM phosphate buffer pH 2.0 and acetonitrile (7:3, v/v). The chromatography was carried out at flow rate of 1.0 mL/min and at room temperature.

Chemical Stability. The chemical stability of the cyclic phosphate prodrugs **1**−**4** was studied in 50 mM (ion strength 0.15) buffered aqueous solutions (HCl buffer at pH 1.0, acetate buffer at pH 5.0, phosphate buffer at pH 7.4, and borate buffer at pH 9.0) 37 °C for three weeks. The incubation mixture was prepared by dissolving the prodrug stock solution (10 mM) in ethanol to preheated buffer solutions. The final prodrug concentration in the incubation mixtures was 200 μM and the ethanol concentration 2%. The samples were withdrawn at appropriate intervals, and acetonitrile was added to the samples (1:1, v/v) to minimize further hydrolysis prior to HPLC analyses. The hydrolysis followed first-order kinetics, and the pseudo-first-order half-lives ($t_{1/2}$) for the hydrolysis of the prodrugs were calculated from the slope of the linear portion of the plotted logarithm of remaining prodrug versus time.

***In Vitro* Bioconversion.** The bioactivation of the cyclic phosphate prodrugs **1**−**4** was determined in isolated rat liver microsomes prepared by standard differential ultracentrifugation and stored at −80 °C until use.[19] The incubation mixture

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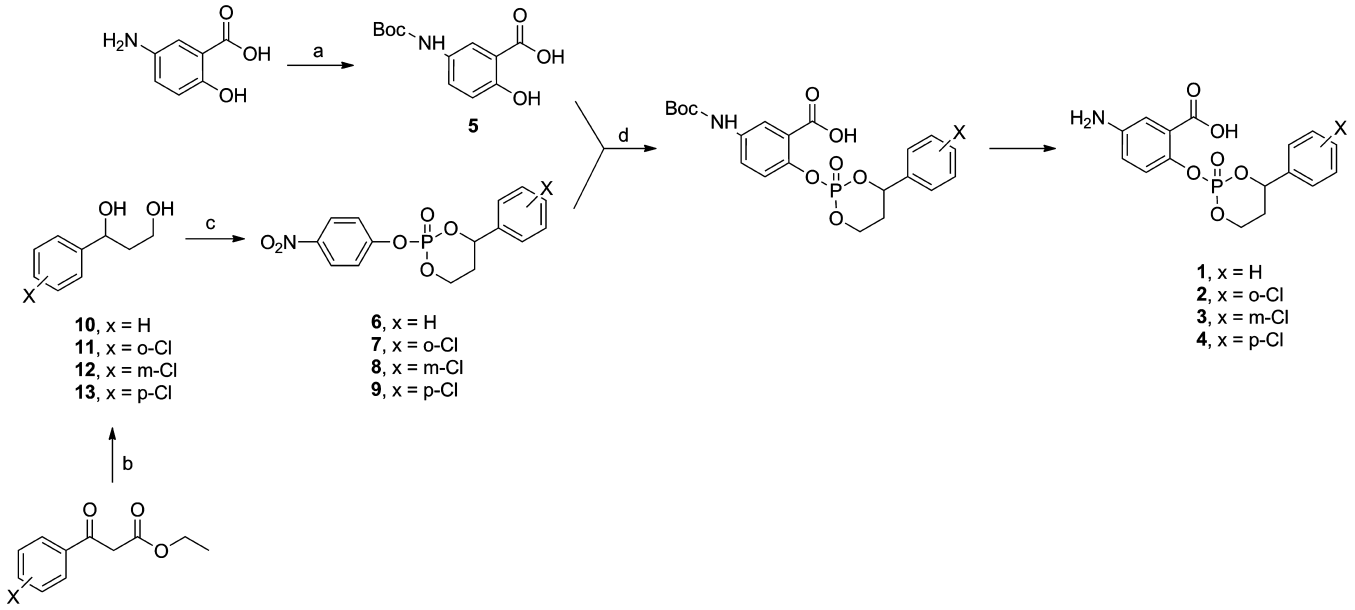
Scheme 1. Synthesis of Cyclic Phosphates of 5-ASA[a]



[a]Reagents and conditions: (a) di-*tert*-butyl dicarbonate, $NaHCO_3$, DMF, + 50 °C, 20 h, 80%; (b) $LiAlH_4$, Et_2O, reflux, 6−8 h, 33−98%; (c) 4-nitrophenyl phosphorodichloridate, Et_3N, THF, 0 °C to rt, 3−4 h, 83−99%; (d) (1) 1 M *tert*-BuMgCl, DMF, rt, 20 h, (2) 3 M HCl (aq), rt, 1−2 h, 33−50%.

was prepared by mixing 1 M Tris-HCl buffer (pH 7.4) and 100 μg of microsomal protein to water and preincubating the mixture for 2 min at 37 °C. Prodrug stock solutions (5 mM) in 50% ethanol (the final concentration of prodrug **1** was 100 μM and the ethanol concentration 1%) and a NADPH regenerating system (1.15 mM NADP, 12.5 mM isocitric acid, 56.25 mM KCl, 187.5 mM Tris-HCl, 12.5 mM $MgCl_2$, 0.0125 mM $MnCl_2$, and 0.77 μL/mL isocitric acid dehydrogenase, pH 7.4) were added, and the mixture was incubated approximately 6 to 24 h at 37 °C. In blank solutions, liver microsomes or the NADPH regenerating system was replaced with the same volume of water. The samples were withdrawn at appropriate intervals, and the enzymatic reactions were terminated by the addition of ice-cold acetonitrile (1:1, v/v). The samples were centrifuged for 15 min at 12000*g* and kept on ice until the supernatants were analyzed by HPLC. The first-order half-lives ($t_{1/2}$) for the reaction of the prodrugs were calculated from the slope of the linear portion of the plotted logarithm of remaining prodrug versus time.

Hydrolysis in alkaline phosphatase solution was determined by incubating a mixture of the cyclic phosphate prodrugs **1**−**4** in ethanol (1 mM), 1 M Tris-HCl buffer (pH 7.4), and alkaline phosphatase EC 3.1.3.1 (type VII-S: from bovine intestinal mucosa, 2.590 units/mg protein) purchased from Sigma (St. Louis, MO, USA) in water at 37 °C for 24 h. The final concentration of the prodrugs was 100 μM and the ethanol concentration 1%, and the incubation mixture contained ca. 100 mU of alkaline phosphatase. In blank solutions, alkaline phosphatase was replaced with the same volume of water. The samples were withdrawn at appropriate intervals, and the enzymatic reactions were terminated by the addition of ice-cold acetonitrile (1:1, v/v). The samples were centrifuged for 15 min at 12000*g* and kept on ice until the supernatants were analyzed by HPLC.

RESULTS AND DISCUSSION

As previously described, the desired CYP-catalyzed prodrug cleavage of cyclic phosphates requires a C4-aryl ring attached to the six-membered heterocyclic ring.[10,14] The benzylic carbon renders the cyclic phosphate more susceptible to CYP3A-mediated oxidation. This makes the cyclic phosphates highly selective prodrugs, since they are bioactivated only inside the cells that are rich in CYP3A forms. Therefore, cyclic phosphate derivatives are ideal for targeting drugs to tissues expressing CYP3As. Although CYP3A forms reside predominantly in the liver, they are also abundantly expressed in the small intestine and with lower levels in the colon.[20−22] Lately, CYP enzymes have become widely exploited in prodrug research. CYPs account for up to 75% of all enzymatic metabolism of drugs, with a predominant role played by the CYP3A4 form. Numerous clinically important drug−drug interactions occur through inhibition or induction of CYPs, especially CYP3A4. In addition, genetic polymorphisms in CYP genes have a significant effect on disposition of a number of commonly used drugs.[23,24] These aspects need to be taken into account when developing prodrugs activated by any specific CYP form, especially CYP3A4.

The cyclic phosphates of 5-ASA, which are taken up into the enterocytes and selectively bioconverted to their corresponding free phosphates, are retained as charged intermediates in the mucosal tissues. This augments the targeting effect and is especially beneficial in Crohn's disease, which is manifested in both small and large intestine.[25] In mucosal tissues the phosphate intermediates are subsequently bioconverted to 5-ASA by intestinal alkaline phosphatase (IAP). Although IAP is mainly expressed in the apical membrane of enterocytes, it is known to be released to the luminal side by lysophosphatidylcholine.[26] Therefore, increased mucosal 5-ASA concentrations could be achieved by the cyclic phosphate prodrug approach.

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Erion et al. have reported that several electron withdrawing substituents, like chlorine in the C4-aryl ring or pyridine as an aromatic ring, augment the prodrug conversion rate, while electron donating substituents lower the rate of bioactiation.[14−16] Although electron donating substituents may increase oxidation rate, the slow β-elimination makes the total bioconversion rate much slower than with electron withdrawing substituents, which in contrast may decrease oxidation rate, but have much faster β-elimination and total bioconversion rate. On the basis of this information four cyclic phosphate prodrugs of 5-ASA were designed and synthesized in the present study (Scheme 1): one with a plain C4-phenyl ring (**1**) and three with a chlorine substituent in the ortho-, meta- and para-positions of the C4-phenyl ring (**2**−**4**). The cyclic phosphates of 5-ASA were prepared by protecting the amino group of 5-ASA with *tert*-butyloxycarbonyl group (*t*-Boc) in basic conditions and then reacting the obtained compound **5**[13] with cyclic phosphate nitrobenzenes **6**−**9** in the presence of *tert*-butylmagnesium chloride (Scheme 1). The *t*-Boc-protecting group was finally cleaved by treatment with aqueous HCl to obtain the cyclic phosphates **1**−**4** with moderate to good yields. As previously described, the cyclic phosphate nitrobenzenes **6**−**9** were prepared by reducing commercial β-keto esters with lithium aluminum hydride ($LiAlH_4$) to provide racemic mixtures of diols **10**−**13**, which were then reacted as unseparated mixtures with 4-nitrophenyl phosphorodichloridate in the presence of triethylamine to yield the compounds **6**−**9**.[14−18] Unseparated diastereomers of cyclic phosphates **1**−**4** were used to evaluate the nonenzymatic and enzymatic stability as well as primary enzymatic bioconversion by CYP enzymes.

Due to the amphoteric properties of 5-ASA and its derivatives, it was challenging to develop a proper HPLC method to evaluate these compounds.[27] Especially 5-ASA phosphate (the intermediate of the prodrug bioconversion process) had poor retention in reverse-phased columns, and when using ion-pair reagents or a normal-phase column the peak symmetries were unsatisfactory. As free phosphates are well-known to be hydrolyzed by ubiquitous phosphatases[28] and since we have previously shown that these cyclic phosphates are bioconverted in two steps, first by CYP enzymes and then by phosphatases,[10] we did not quantify the free phosphate, only 5-ASA, the end product of the total prodrug bioconversion process. Agilent Zorbax ODS analytical column (250 mm × 4.6 mm, 5 μm) was found to produce the most satisfactory chromatographic separation of the prodrugs **1**−**4** and 5-ASA by isocratic elution of 50 mM phosphate buffer pH 2.0 and acetonitrile (7:3; 1.0 mL/min). The absorption maximum for all cyclic phosphates was near to nonspecific wavelength, 200 nm, which in its part complicated the detection of these compounds by UV. Moreover, none of the cyclic phosphates emitted fluorescent light, unlike 5-ASA, which was detected by the fluorescence detector (λ_{exc} = 310 nm, λ_{emiss} = 500 nm) in our studies. Therefore, mass spectrometry should be used, if possible, to detect these compounds in further studies.

Chemical stability of the prodrugs **1**−**4** was studied in 50 mM aqueous buffer solutions at pH values ranging from 1.0 to 9.0 at 37 °C. When hydrolyzed, the degradation of prodrugs followed pseudo-first-order kinetics and released stable ring-opened forms (Figure 2). The rate of hydrolysis was remarkably faster with the para-substituted prodrug **4** than with meta- and ortho-substituted prodrugs **3** and **2**, respectively, whereas the prodrug **1** did not hydrolyze at all during the three weeks' experimental time (Table 1). The

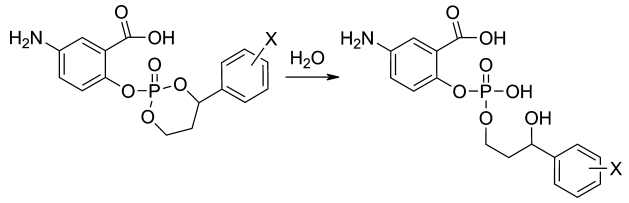

Figure 2. Proposed hydrolysis reaction of cyclic phosphates of 5-ASA in buffer solutions.

electronegative chlorine most probably weakened the bond between phosphorus and oxygen atoms after the nucleophilic attack of the water molecule to the phosphorus atom, which led to the formation of a ring-opened product. These products were identified on the basis of ^{1}H, ^{13}C, and ^{31}P NMR spectra (data not shown). Since the hydrolysis produced stable ring-opened forms, which did not release any 5-ASA, it can be concluded that the bioactivation reaction can only occur in the presence of prodrug activating enzymes. Although the chlorine substituent in the C4-aryl ring lowered the chemical stability of cyclic phosphates of 5-ASA and at least the cyclic phosphate **4** was considered to be too unstable for targeting purposes, all the prodrugs were selected for the bioactivation studies to evaluate how the position of this electron withdrawing group affects the bioconversion process.

Enzymatic stability of the prodrugs **1**−**4** toward hydrolysis by alkaline phosphatase (from bovine intestinal mucosa) was studied to confirm that these cyclic phosphates were not susceptible for possible premature bioconversion by IAP. As shown in Table 2, all four prodrugs were highly stable in intestinal alkaline phosphatase solution at 37 °C and obviously poor substrates for phosphatases. Since these experiments were carried out in Tris-HCl buffer at pH 7.4, it clearly shows that phosphate ions increase the rate of hydrolysis in aqueous media (Table 1).

Bioactivation of the prodrugs **1**−**4** was studied as previously described with isolated liver microsomes known to be rich in CYP enzymes.[10] Comparison of the bioconversion rates in untreated rat liver microsomes (control) with liver microsomes obtained from rats treated with the CYP3A-inducing agent, dexamethasone, gave us initial information whether these prodrugs behaved similarly as the cyclic phosphates published previously, i.e., these prodrugs were activated mainly by CYP3A forms. Unexpectedly, the cyclic phosphate **1** was not oxidized in the rat liver microsomes at all (Table 2), although it has been reported that a simple phenyl ring at the C4-position of the cyclic phosphate should fulfill the requirements for the CYP-catalyzed oxidation.[10,14] In contrast, the cyclic phosphates **2**, **3**, and **4** with a chlorine substituent at the ortho-, meta- and para-positions, respectively, were oxidized in rat liver microsomes (Table 2). Hence, in the case of 5-ASA an aromatic activating substituent is definitely needed to obtain the desired bioconversion of the cyclic phosphates. The bioconversion reaction of the prodrugs **2**−**4** followed first-order kinetics and released small amounts of 5-ASA. The reason why only low levels of 5-ASA (10−60%) were detected in our experiments was most probably due to other cellular reactions, such as *N*-acetylation, which produced other derivatives of 5-ASA that were not monitored in our experiments. Since the reaction did not occur in the blank samples (without microsomes or NAPDH, an essential component for the CYP-catalyzed reactions), we concluded that the reaction was CYP-mediated.

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Table 1. Half-Lives of the Cyclic Phosphate Prodrugs 1−4 in 50 mM Buffer Solutions at 37 °C (Mean ± SD, n = 3)

	$t_{1/2}$ (h)			
compd	pH 1.0	pH 5.0	pH 7.4	pH 9.0
1	*a*	*a*	*a*	*a*
2	88.73 ± 5.23	91.96 ± 11.82[b]	75.13 ± 13.37	82.60 ± 8.05
3	4.69 ± 0.04	4.14 ± 0.08	5.78 ± 0.50	5.57 ± 0.20
4	0.48 ± 0.20	0.58 ± 0.14	0.98 ± 0.32	0.56 ± 0.34

[a]No degradation was observed during the three weeks' incubation. [b]n = 2.

Table 2. Half-Lives of the Cyclic Phosphate Prodrugs 1−4 in Rat Liver Microsomes (RLM), Dexamethasone-Pretreated Rat Liver Microsomes (DEX), and Bovine Intestinal Alkaline Phosphatase Solution (IAP) at 37 °C (Mean ± SD, n = 3)

	$t_{1/2}$ (min)		
compd	RLM	DEX	IAP
1	*a*	*a*	*a*
2	702.28 ± 265.44	455.44 ± 135.57	*a*
3	292.50 ± 41.70	156.90 ± 31.50	*a*
4	23.03 ± 1.06	26.65 ± 0.71	*a*

[a]No degradation was observed during the 24 h incubation.

The enzymatic reaction rates were much faster with the cyclic phosphate **4** than with the cyclic phosphates **3** and **2**, which strongly implies that the chlorine substituent at the para-positions speeds up the oxidation rate remarkably. As expected, the cyclic phosphates **2** and **3** were oxidized more rapidly in dexamethasone-induced rat liver microsomes, which indicates that CYP3A forms were involved in the bioactivation process (Figure 3). However, in the case of the para-substituted

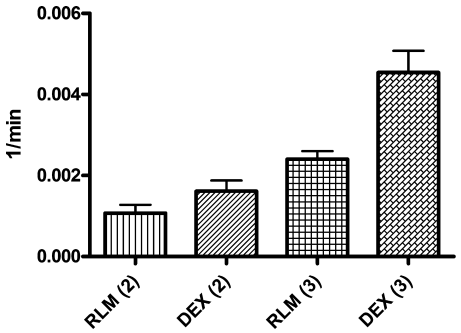

Figure 3. First order reaction rate constants of the enzymatic reaction of the prodrugs **2** and **3** in rat liver microsomes (RLM) and dexamethasone-induced rat liver microsomes (DEX).

prodrug, no difference was seen between the reaction rates in control rat liver microsomes and dexamethasone-induced ones (Table 2). Most likely the oxidation of the cyclic phosphate **4** was mediated by other CYP forms or other enzymes. Together with low chemical stability, the cyclic phosphate **4** was considered as an unideal prodrug for targeting of 5-ASA. In contrast, the cyclic phosphate **3** with chlorine at the meta-position in the C4-aryl ring seems to have both reasonable bioactivation rate and sufficient chemical stability to target 5-ASA to the mucosal tissues of intestine, while the ortho-substituted cyclic phosphate **2** may have too slow a bioconversion rate. However, both of these cyclic phosphate prodrugs of 5-ASA should be carried on to future studies.

CONCLUSION

In conclusion, the results of the present study are consistent with the earlier ones, i.e., drugs having a free hydroxyl group can be converted into cyclic phosphates that can serve as CYP3A-activated prodrugs. In the case of cyclic phosphates of 5-ASA, an unsubstituted phenyl prodrug was found to be inert to CYP-mediated bioactivation and, therefore, a ring activating substituent has to be incorporated to the phenyl ring of the cyclic phosphate to achieve the desired CYP-catalyzed oxidation. However, different ring activating substituents may have a strong impact not only on the bioactivation rate but also on the chemical stability of these prodrugs, and therefore an appropriate balance between the rates of bioactivation and chemical stability needs to be taken into consideration when adding an electron withdrawing substituent to the aromatic ring of the cyclic phosphates. Based on this study, the cyclic phosphates of 5-ASA with a chlorine substituent in the ortho- or meta-position of the C4-aryl ring may be suitable for further evaluation to deliver higher local concentrations of 5-ASA to the mucosal cells, especially to the small intestine.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

5-ASA, 5-aminosalicylic acid (mesalamine); CYP, cytochrome P450 enzymes; IAP, intestinal alkaline phosphatase

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