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Synthesis of mevalonate- and fluorinated mevalonate prodrugs and their *in vitro* human plasma stability

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ABSTRACT

The mevalonate pathway is essential for the production of many important molecules in lipid biosynthesis. Inhibition of this pathway is the mechanism of statin cholesterol-lowering drugs, as well as the target of drugs to treat osteoporosis, to combat parasites, and to inhibit tumor cell growth. Unlike the human mevalonate pathway, the bacterial pathway appears to be regulated by diphosphomevalonate (DPM). Enzymes in the mevalonate pathway act to produce isopentenyl diphosphate, the product of the DPM decarboxylase reaction, utilize phosphorylated (charged) intermediates, which are poorly bioavailable. It has been shown that fluorinated DPMs (6-fluoro- and 6,6,6-trifluoro-5-diphosphomevalonate) are excellent inhibitors of the bacterial pathway; however, highly charged DPM and analogs are not bioavailable. To increase cellular permeability of mevalonate analogs, we have synthesized various prodrugs of mevalonate and 6-fluoro- and 6,6,6-trifluoromevalonate that can be enzymatically transformed to the corresponding DPM or fluorinated DPM analogs by esterases or amidases. To probe the required stabilities as potentially bioavailable prodrugs, we measured the half-lives of esters, amides, carbonates, acetals, and ketal promoieties of mevalonate and the fluorinated mevalonate analogs in human blood plasma. Stability studies showed that the prodrugs are converted to the mevalonates in human plasma with a wide range of half-lives. These studies provide stability data for a variety of prodrug options having varying stabilities and should be very useful in the design of appropriate prodrugs of mevalonate and fluorinated mevalonates.

1. Introduction

The mevalonate pathway (Fig. 1) is an important cellular metabolic pathway present in all higher eukaryotes and many bacteria. Isopentenyl diphosphate (IPP), an intermediate in this pathway, is an important precursor of isoprenoids, which leads to many biologically active small molecules, including cholesterol, steroid hormones, and vitamin A [1]. Therefore, it is not surprising that enzymes in the mevalonate pathway are targets for a variety of drug discovery programs [2]. The statin cholesterol-lowering drugs target 3-hydroxy-3-methylglutaryl CoA reductase, the enzyme that produces mevalonate [3]; the osteoporosis drug alendronate inhibits the synthesis of farnesyl diphosphate from IPP [4]; enzymes in the mevalonate pathway are also targeted for cancer [5] and parasites [6]. Leyh and co-workers discovered that the mevalonate pathway in *Streptococcus pneumoniae* is regulated by 5-diphosphomevalonate (DPM) [7]. They showed that DPM is a feedback inhibitor of mevalonate kinase (MK), and binds tightly to an allosteric site [8] of the pneumococcal MK. However, human MK is not inhibited by DPM at concentrations that effectively inhibit the S. *pneumoniae* system [9]. Therefore, DPM can be a lead compound for the development of new anti-pneumococcal antibiotics that do not perturb human metabolism.

Abbreviations: DMP, diphosphomevalonate; MK, mevalonate kinase; PMK, phosphomevalonate kinase; DPM, 6-fluoro- and 6,6,6-trifluoro-5-diphosphomevalonate; DPM-DC, diphosphomevalonate decarboxylase; IPP, isopentenyl diphosphate; EDCI, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride; HBTU, *N*,*N*,*N*,*N*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate; MIC, minimal inhibitory concentration; THB, tissue homogenization buffer.

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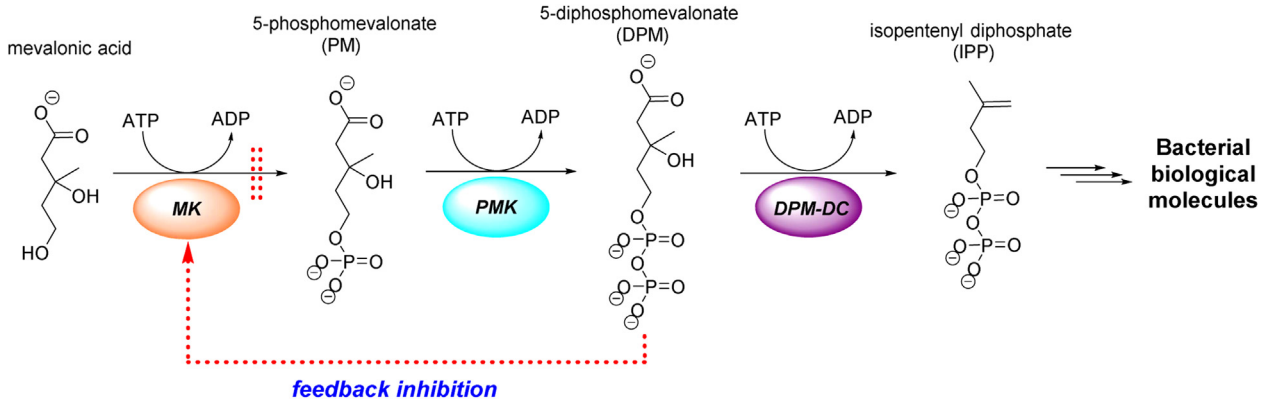

Fig. 1. The bacterial mevalonate pathway. Conversion of mevalonic acid to isopentenyl diphosphate occurs in three ATP-dependent steps. DPM is a feedback inhibitor of MK: MK, mevalonate kinase; PMK, phosphomevalonate kinase; DPM-DC, diphosphomevalonate decarboxylase.

In early studies of DPM analogs [10], it was found that 6,6,6-trifluoromevalonate was converted into the corresponding diphosphate enzymatically, which inhibited DPM decarboxylase (DPM-DC) and led to the accumulation of DPM in rat liver homogenates [11]. Moreover, 6-fluoromevalonate causes the accumulation of phosphorylated mevalonates and completely blocks the related bioactivity of mevalonate at 200 μM concentration [12]. However, whereas a functional mevalonate pathway is essential for the survival of bacteria, suppression of this pathway in humans results in minimal side effects, as evidenced by the common use of statin drugs, which block cholesterol biosynthesis at a step prior to DPM-DC, and by antiproliferative drugs, such as bisphosphonates, which block farnesyltransferase [13]. Furthermore, antibacterial treatment is short in duration, which should not have a serious effect on the products of this pathway. Nonetheless, diphosphate compounds are generally not suitable for use as drugs; because of their highly charged structure (4-), they are not expected to penetrate the negatively charged bacterial cell membrane [14]. Also phosphatases can degrade the diphosphate group easily.

Because of the importance of mevalonate and phosphorylated metabolites to drug discovery, neutral and less polar prodrugs, chemically modified molecules of the pharmacologically active moiety that are transformed into the active form *in vivo* [15], were designed to avoid these potential bioavailability problems. The charged carboxylic acid was protected as an ester, lactone, or amide to make it neutral. To explore the influence of polarity of the prodrug on human absorption and bacterial cell permeation, the two hydroxyl groups of the mevalonate were converted to carbonate, acetal, and ketal prodrugs. These esters [16], lactones [17], amides [18], carbonates [19], acetals [20,21], and ketals [22], having half-lives ranging from a couple of minutes to several days in human plasma, were chosen as the promoieties of the carboxyl and hydroxyl functionalities of mevalonate. These analogs should be enzymatically hydrolyzed to their original mevalonate or fluorinated mevalonates in both humans and bacteria, and then the fluorinated mevalonates can be enzymatically converted to the phosphorylated forms [23]. The stabilities of diverse analogs (Fig. 2) in human blood plasma were studied to develop an armamentarium of promoieties for further *in vitro* and *in vivo* studies.

2. Results and discussion

2.1. Chemistry

Cyclic carbonate prodrug **4** was prepared from mevalonolactone (**1**) using the synthetic route described in Scheme 1. The hydrolysis of **1** with aqueous KOH afforded a solution of **2**, which was neutralized to pH 7-8 with aqueous HCl and lyophilized to remove water. If neutralization was not carried out, starting material **1** was regenerated during lyophilization. The crude carboxylic acid (**2**) was converted to the corresponding benzyl ester (**3**) via treatment with benzyl bromide and tetrabutylammonium bromide. Although a portion of ester **3** was converted to the starting material (**1**) during column chromatography with silica gel, **3** was isolated as the major product (69% yield). Ester **3** was easily converted to the cyclic carbonate (**4**) via treatment with triphosgene. An alternative route to the benzyl ester (**3**) is also shown in Scheme 1; TBS protection of the hydroxyl group of 4-hydroxy-2-butanone (**5**), followed by an aldol reaction with benzyl acetate using LDA, afforded **7** in excellent yields. Deprotection of the TBS group in compound **7**, with tetrabutylammonium fluoride and two equivalents of acetic acid at 0 °C, afforded desired alcohol **3**. The reaction of crude product **3** with triphosgene yielded cyclic carbonate **4;** lactone **1** was still generated gradually before **3** disappeared completely.

Cyclic carbonate analogs **9a**,**b** and **10a-c** were synthesized from benzyl ester **4** after removal of the benzyl group via palladium-catalyzed hydrogenolysis (Scheme 2). The coupling reaction of carboxylic acid **8** with various phenols and amines using EDCI or HBTU provided the desired esters (**9a**,**b**) and amides (**10a-c**) in moderate to good yields (Scheme 2). The obtained benzyl amide derivatives were expected to be more stable than the ester derivatives in plasma. To confirm the relative stability of other less stable amides (due to the electron withdrawing effect), a phenyl amide (**10a**) and a 4-fluorobenzyl amide (**10c**) were prepared.

The synthetic route for the 6-fluoromethyl cyclic carbonate analog is shown in Scheme 3. The addition of allylmagnesiun bromide (1.95 equiv) to ethyl fluoroacetate **11** at 0 °C for 30 min afforded diolefin **12**. This reaction was sensitive to the duration and the equivalents of Grignard reagent; an addition of excess allymagnesium bromide or prolonged reaction times resulted in undesired side product generation. Ozonolysis of crude product **12**, followed by oxidation with H_2O_2 gave dicarboxylic acid **13**. The benzylation of crude product **13** was conducted to give diester **14** in more than 50% yield for four steps. Partial reduction of **14** with DIBAL-H (3–4 equiv) at 0 °C in THF afforded the desired compound (**15**). Because **15** easily underwent intramolecular lactone formation on silica gel, the crude mixture was allowed to react with triphosgene without further column purification to obtain cyclic carbonate **16** in moderate yields. The benzyl deprotection of **16**, followed by esterification with 4-fluorobenzyl bromide, or 2,4-difluorobenzyl bromide, provided the corresponding esters (**18a-b**), respectively.