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Deuterated Clopidogrel Analogues as a New Generation of Antiplatelet Agents

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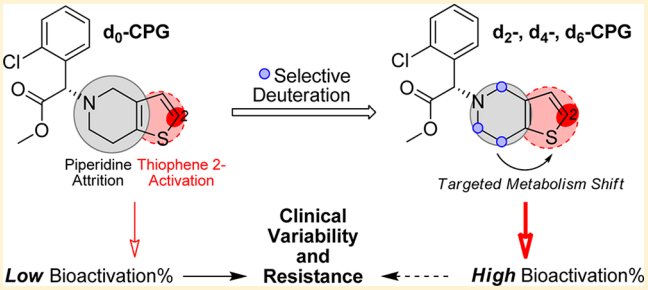
Supporting Information

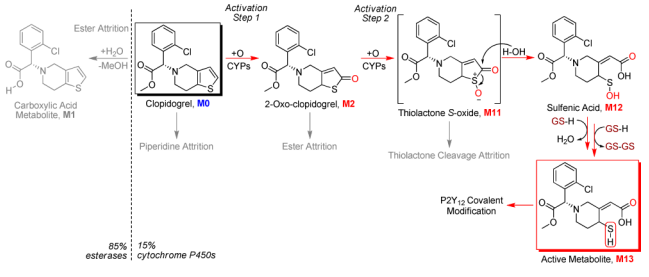
ABSTRACT: Clopidogrel (CPG) is an antithrombotic prodrug that needs hepatic cytochrome P450 (CYP) enzymes for its bioactivation. The clinical effects of CPG have been associated with high intersubject variability and a certain level of resistance. Recently, comprehensive biotransformation studies of CPG support that the observed clinical uncertainty stems from the low bioactivation efficiency, which is attributed to extensive attritional metabolism (e.g., hydrolysis of the methyl ester functionality and oxidation of the piperidine moiety). With the goal of potentiating the desired thiophene 2-oxidation through minimal structural modification, we have adopted the strategy of *targeted metabolism shift* and have designed and synthesized deuterated piperidine analogues of CPG. In vitro studies showed that the prodrug activation percentages have been significantly increased for the deuterated analogues as a result of stability enhancement of the piperidine moiety. In a pharmacological study with a rat model, oral administration of the deuterated analogues also demonstrated higher inhibitory activity than that of CPG against adenosine diphosphate (ADP) induced platelet aggregation. These deuterated analogues represent a new generation of antiplatelet agents with the potential to overcome the major clinical drawbacks of CPG.



KEYWORDS: *Clopidogrel resistance, clinical variability, prodrug attrition, piperidine deuteration, targeted metabolic shift, bioactivation potentiation*

Clopidogrel (CPG) is a thienopyridine antiplatelet prodrug that has been widely used in the treatment of cardiovascular diseases, including atherothrombosis, unstable angina, and myocardial infarction.[1] As shown in Scheme 1, CPG (M0) is activated through a two-step cytochrome P450 (CYP)-catalyzed process to form its active metabolite (M13).[2,3] Despite being one of the most prescribed cardiovascular medications of the past decade, CPG is associated with a high clinical uncertainty for its antithrombotic therapy. It has been reported that 20−40% of patients that receive the drug showed poor or no response to it.[4,5]

Scheme 1. Metabolic Activation and Major Attrition Pathways of CPG



To address the observed clinical variability and resistance, the chemical mechanism of CPG bioactivation has been under extensive investigation. Genetic factors that can impact the therapeutic outcomes are being sought for the design of personalized prodrug treatments. It has been hypothesized that the active metabolite formation from CPG is dependent on genetic polymorphic enzymes, and this leads to the observed intersubject variability. However, research results have shown that both steps of the prodrug activation are catalyzed by various CYPs, and genetic polymorphic enzymes such as paraoxonase-1 (PON-1) or CYP2C19 do not play deciding roles in catalyzing the active metabolite formation.[6−9]

Our recent research has revealed that the first step of CPG bioactivation, 2-oxidation of the thiophene motif leading to M2 formation, is significantly attenuated by CYP3A4/5-catalyzed oxidation of the nonactivating piperidine motif and the

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thiophene 3-position,[8] in addition to the previously reported methyl ester hydrolysis.[10,11] Subsequently, we studied the second step and found that the formation of M13 from M2 is accompanied by multiple attritional pathways as well, which further attenuate the active metabolite formation.[12] On the basis of these discoveries, the overall percentage of bioactivation, conversion from M0 to M13, is expected to be much lower than previously presumed (i.e., 1% or lower). Consequently, the vulnerably low plasma exposure of M13 is likely to be significantly impacted by multicomponent factors (e.g., levels of attenuating enzymes, individual oxidative stress, off-target hepatic and plasma proteins), leading to the varying levels of therapeutic response and elusive clinical correlations.[8]

This slim bioactivation, on the other hand, demonstrates the high potency of the active metabolite against its antiplatelet target $P2Y_{12}$,[13] as well as a high ligand efficiency of CPG (MW = 321), both resulting from the receptor covalent modification. This mode of pharmacological action also poses a barrier to medicinal chemists in discovering better analogues, because the low chemical flexibility of CPG, toward both the bioactivating enzymes and its molecular target, is unlikely to accommodate major structural changes. In addition, the high chemical reactivities of the active metabolite (M13) and its thiolactone *S*-oxide precursor (M11) confer CPG with a narrow therapeutic window,[14] as will overdosing not only aggravate the adverse effects of hemorrhage but also lead to severe drug−drug interactions.[15−17] On the basis of these understandings, our medicinal chemistry endeavor was set to achieve bioactivation potentiation through minimal structural change, with possibly taking advantage of the safety records that CPG has established over its long clinical use. The strategy we adopted is targeted metabolism shift based on selective deuteration.

It has been reported that the absorbed CPG of an oral dose undergoes esterase catalyzed hydrolysis to form its inactive carboxylic acid metabolite (M1), which accounts for about 85% of prodrug loss.[11] Our earlier studies have shown biotransformations of the remaining 15% partition between the piperidine moiety and the thiophene moiety, as mediated by CYP3A4/5 and multiple CYPs, respectively; the bioactivating thiophene 2-oxidation is attenuated by extensive piperidine metabolism.[8] Our results have also confirmed that selective inhibition of piperidine metabolism can significantly potentiate the desired thiophene 2-activation. On the basis of these findings, together with the structural analyses of the attritional metabolites, we designed deuterated piperidine analogues of CPG (d_0-CPG), namely, d_2-CPG, d_4-CPG, and d_6-CPG (Figure 1).

The chemical synthesis of the CPGs is anchored by a Mannich condensation reaction.[18] As shown in Scheme 2A, d_0- (**1**) and d_2-CPG (**2**) were prepared from reactions of the commercially available CPG-I intermediate (**10**) with paraformaldehyde and d_2-paraformaldehyde, respectively. To obtain d_4- (**3**) and d_6-CPG (**4**) (Scheme 2B), the deuterated intermediate, d_4-CPG-I (**11**), was first synthesized through a coupling reaction between amine intermediate **10** and deuterated tosylate intermediate **8.** The latter was prepared in three steps with full deuteration at its two methylene positions (Scheme 2C). The final CPGs were converted to their hydrochloride salts for in vitro and in vivo evaluations.

In vitro bioactivation studies of CPGs were conducted in parallel in both human and rat liver microsomes (HLM and RLM). Incubation conditions were adopted from the previously reported M0-to-M2 formation for CPG.[8] The turnovers of the parent compounds (ΔM0) and the amounts of the formed M2 from incubations of the four CPGs were quantified; the bioactivation percentages were calculated as "M2/ΔM0". As shown in Figure 2, in HLM, the formations of M2 accounted

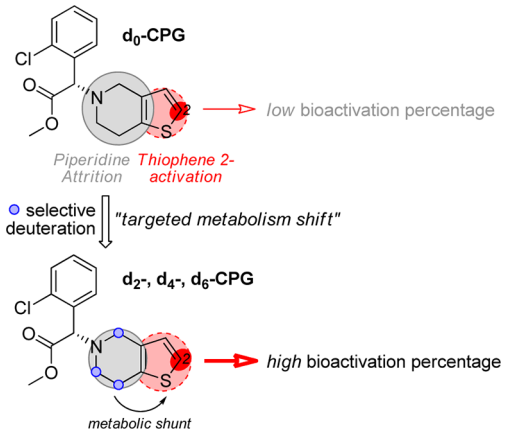
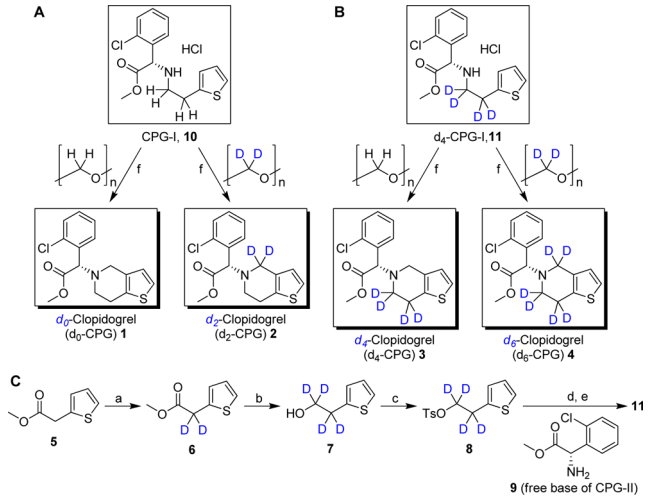


Figure 1. Structural modification strategy and the design of the deuterated CPG analogues.

Scheme 2. Synthesis of CPG and Its Deuterated Analogues[a]



[a]Reagents: (a) NaOMe, MeOD, 91%; (b) $NaBD_4$, MeOD, THF, 70 °C, 95%; (c) *p*-TsCl, Et_3N, CH_2Cl_2, 97%; (d) $KHCO_3$, CH_3CN, 80 °C; (e) HCl, Et_2O, 76% (two steps).; (f), HCl, H_2O, 80 °C, 90%. [b]CPG-II is the commercially available L-tartaric acid salt of **9**. [b]CPG-II is the commercially available L-tartaric acid salt of **9**.

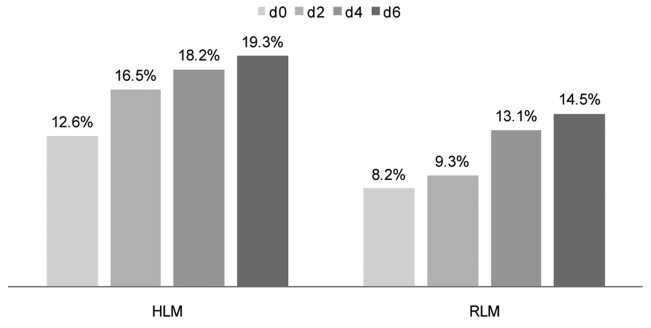


Figure 2. Bioactivation (M2 formation from M0) percentages (out from the CYP catalyzed conversions) of the CPGs in human and rat liver microsomes (HLM and RLM).

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for higher percentages of all the CYP catalyzed conversions of M0 for d_2-, d_4-, and d_6-CPG than for CPG (d_0-CPG). In RLM, the deuterated analogues also exhibited higher bioactivation efficiencies with a pattern that is commensurate with the metabolic profiles of CPG. These results show that, compared to CPG, the deuterated analogues yield significantly more pharmacologically desired metabolites and less attritional ones, attributable to the reduced metabolism on the nonactivating piperidine moiety. The overall conversion rates of the CPGs are illustrated in Figure 3. In contrast to the significant boost of the

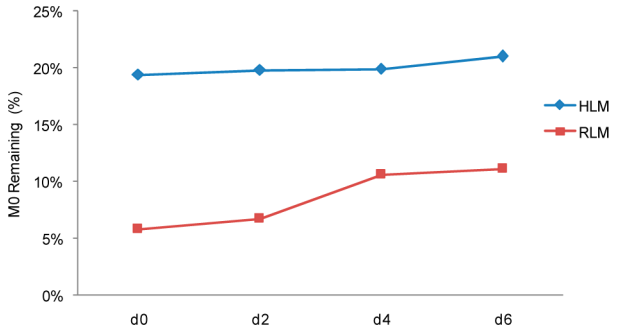

Figure 3. Metabolic stabilities of the CPGs in human and rat liver microsomes (HLM and RLM).

M2 formation, the metabolic stabilities of the parent compounds showed little changes in HLM and marginal changes in RLM with the deuterated analogues. These results confirm the successful utilization of the strategy of targeted metabolism shift: selective deuteration enhances the stability of the nonactivating moiety and shunts metabolism to the desired fragment, leading to bioactivation potentiation (Figure 1).

The deuterated analogues were compared with CPG in vivo for their activities against ADP-induced platelet aggregation. In a rat model similar to the one that has been reported previously,[19] CPGs were orally administered (10 mg/kg), and the ADP-induced platelet aggregation was determined by Born's method.[20] From the obtained kinetic curves, the maximal aggregation and the aggregation after 5 min of ADP induction were obtained and are summarized in Table 1. Both

Table 1. Inhibitory Effects of the CPGs on the ADP-Induced Platelet Aggregation in Rats at a Dose of 10 mg/kg[a]

	aggregation (%)	
group	max.	5 min
vehicle	76.0 ± 7.8	69.8 ± 10.9
d_0	60.4 ± 9.0*	29.4 ± 18.8*
d_2	50.4 ± 13.1*	12.9 ± 13.8*
d_4	49.1 ± 11.9*$^{\#}$	7.3 ± 9.0*$^{\#\#}$
d_6	50.6 ± 13.3*	7.0 ± 12.8*$^{\#}$

[a]Aggregation measurements 2 h after oral administration. *$P < 0.01$ vs vehicle group. $^{\#}P < 0.05$. $^{\#\#}P < 0.01$ vs d_0 group. Data are the mean ± SD, $n = 8$.

sets of results demonstrate that the deuterated analogues exhibit higher antiplatelet activity than CPG. The goal of this early stage in vivo study is to confirm that the bioactivation potentiations observed in vitro translate to potency improvements in an animal model. Due to the subtle nature of the structural modification and the targeted efficacy enhancement, clinical projections of the deuterated analogues require full exploration of the in vitro−in vivo and animal−human correlations. Currently, extensive preclinical testing, including cross-species evaluations and animal-to-human extrapolations, are underway for further development of these deuterated analogues.

The in vitro and in vivo studies presented herein have indicated that the deuterated analogues have the potential to alleviate the major clinical drawbacks of CPG, possibly without altering its long existing antithrombotic regimens, such as the once-a-day low dose and the conjunction use with aspirin. More importantly, the efficacy enhancement is unlikely to jeopardize the clinical safety records of CPG. Currently, to overcome the disadvantages of CPG, substituting therapies are as follows: (1) dose increase of CPG (e.g., doubling), (2) adjunctive use of cilostazol with CPG, and (3) use of other antiplatelet agents, such as prasugrel or ticagrelor.[21] Being closely related to CPG, the improved deuterated analogues are expected to stay in the narrow therapeutic window and be potentially more beneficial than the above alternative treatments. For example, compared to "CPG doubling", the deuterated analogues might yield similar effectiveness but avoid some adverse complications of overdosing. This can increase patient compliance and more safely and effectively manage the cardiovascular risk due to low responsiveness. These deuterated analogues also differ from vicagrel, another CPG based preclinical antiplatelet agent, in their mechanistic approach.[19] The release of M2 from vicagrel is through facile hydrolysis of a masked thiolactone promoiety, which could lead to similar limitations as direct dosing of M2, including fatal bleedings, drug−drug interactions, and other adverse effects.[12] Taken together, the deuterated CPGs represent a new generation of antiplatelet agents with the potential to address unmet medical needs in treating cardiovascular complications, especially the risk of recurrent ischemic events due to poor responsiveness or nonresponsiveness to CPG.

A few general principles for medicinal chemistry have been shaped along with these studies of CPG. First, the relationship between the prodrug design and its treatment consistency is dependent on the bioactivation percentage. Low active metabolite conversion, especially with the involvement of variable attenuating enzymes, is likely to foster clinical uncertainties. Although considered feasible to leverage the intersubject variability, the means of personalized prodrug therapy can be complicated, and often misleading, because of the tangled mechanistic roles of the genotypes. Second, as demonstrated herein, the rational use of deuteration, a seemingly simple medicinal chemistry approach, for drug action enhancement, can be achieved with the understandings of the metabolic fates and the therapeutic profiles of the drug. Third, CPG, with its high ligand efficiency, can serve as a prototype for a promising direction of drug discovery: covalent receptor modification via targeted ligand bioactivation.[22] Although some drugs with such a mode of action, such as omeprazole and capecitabine, have been in clinical use, major hurdles in this field are evident for its development. Systematic methodologies are needed to address major challenges, such as the following: (1) how to take the often pre-excluded chemically reactive fragments for designing drug candidates with such a mode of action; (2) how to explore and exploit the biochemical features in the human body to channel the desired bioactivation.

Recent investigations are beginning to unravel the long overdue biochemical puzzle of CPG. On the basis of the

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previous biotransformation studies and the therapeutic analyses of CPG, medicinal chemistry approaches were carefully designed, and a series of deuterated analogues of CPG were prepared. The in vitro and in vivo studies have confirmed that the subtle structural modification of selective piperidine deuteration yields the targeted metabolic shunt and leads to the desired bioactivation potentiation. These deuterated analogues hold a promise for overcoming the prominent clinical drawbacks of CPG. The research presented here is part of a continuous effort to use CPG as a tool compound to build a methodology of "utilizing the human body to generate its own medicine". The discovery of deuterated CPGs might not only lead to superior antithrombotic medications but, more importantly, could also serve as an example for future chemical therapeutics.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures of CPG synthesis, in vitro bioactivation and in vivo pharmacological studies, and spectral and chromatographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Y.Z. made the initial discovery, designed and synthesized the deuterated CPGs, conducted the in vitro studies, analyzed the results, and wrote the manuscript. J.Z. conducted part of the compound characterization, including HRMS and enantiomeric analyses. B.J. conducted in vivo studies of the CPGs.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CPG, clopidogrel; CYP, cytochrome P450; PON-1, paraoxonase-1; HLM, human liver microsomes; RLM, rat liver microsomes; ADP, adenosine diphosphate

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