Overcoming Clopidogrel Resistance: Discovery of Vicagrel as a Highly Potent and Orally Bioavailable Antiplatelet Agent

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Ⓢ Supporting Information

ABSTRACT: A series of optically active 2-hydroxytetrahydrothienopyridine derivatives were designed and synthesized as prodrugs of clopidogrel thiolactone in order to overcome clopidogrel resistance. The final compounds were evaluated for their inhibitory effect on ADP-induced platelet aggregation in rats. Compound **9a** was selected for further in vitro and in vivo metabolism studies, since its potency was comparable to that of prasugrel and was much higher than that of clopidogrel. Preliminary pharmacokinetic study results showed that the bioavailability of clopidogrel thiolactone generated from **9a** was 6-fold higher than that generated from clopidogrel, implying a much lower clinically effective dose for **9a** in comparison with clopidogrel. In summary, **9a** (vicagrel) holds great promise as a more potent and a safer antiplatelet agent that might have the following advantages over clopidogrel: (1) no drug resistance for CYP2C19 poor metabolizers; (2) lower dose-related toxicity due to a much lower effective dose; (3) faster onset of action.
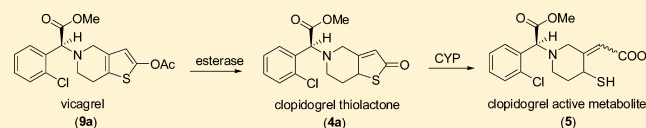
■ INTRODUCTION

Clopidogrel (**1**) (Figure 1) is an oral antiplatelet agent used to prevent blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Currently, dual treatment with aspirin and clopidogrel is the cornerstone of antiplatelet therapy for patients with acute coronary syndrome (ACS) and prevention of thrombotic events after percutaneous coronary intervention (PCI) with stenting.[1] The drug works by
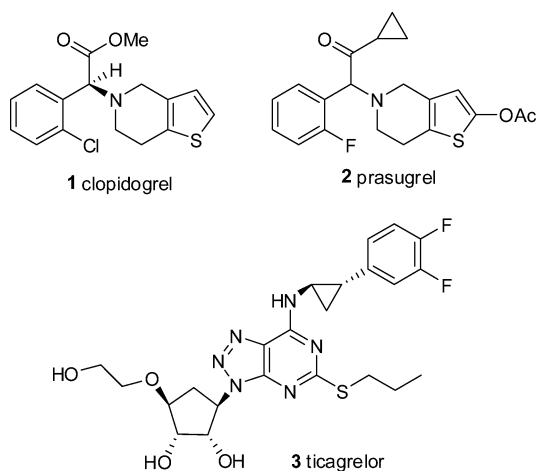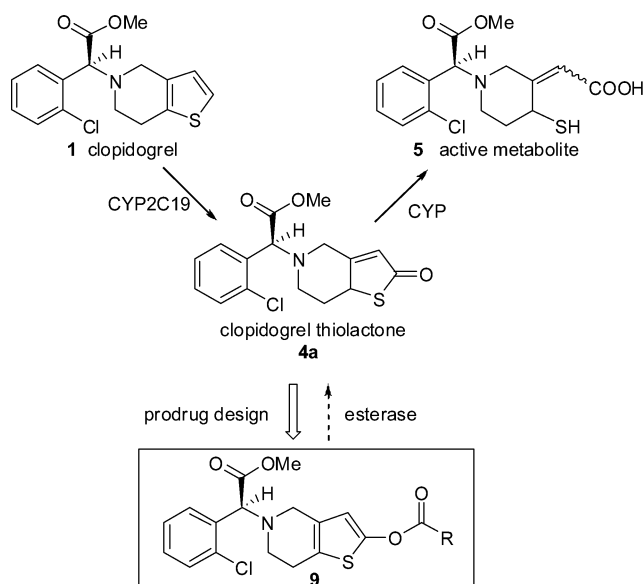
irreversibly inhibiting P2Y12 receptor, a subtype of adenosine diphosphate (ADP) receptors on the platelet membrane. It is a prodrug requiring two-step metabolic conversion by the cytochrome P450 (CYP) system to generate clopidogrel active metabolite (AM, **5**) via clopidogrel thiolactone (**4a**) (Scheme 1).[2] However, nonresponsiveness or poor responsiveness to clopidogrel therapy occurs in up to 30% of Caucasian patients (~2% are poor metabolizers (PMs), ~26% are intermediate metabolizers (IMs)),[3] especially in PMs carrying CYP2C19 2* loss-of-function polymorphism, leading to lower levels of the active metabolite of clopidogrel, less inhibition of platelets, and a 1- to 5-fold higher risk for death, myocardial infarction, and stroke in comparison with noncarriers.[4,5] This refers to the concept of clopidogrel resistance (CR). In 2010, the FDA put a blackbox warning on clopidogrel to make patients and healthcare professionals aware that CYP2C19 PMs are at high risk of treatment failure. Currently, clinical practice to overcome CR includes (1) the adjunctive use of cilostazol, (2) increasing the dose of clopidogrel, (3) the use of new antiplatelet agents, such as prasugrel (**2**) and ticagrelor (**3**) (Figure 1).[6] However, the above approaches may increase the risk of major and fatal bleedings and reduce patient compliance.[7] Taken together, there are still unmet medical needs for the treatment of ACS and its complications, especially

Figure 1. Structures of P2Y12 receptor antagonists.

Scheme 1. Metabolic Activation of Clopidogrel and Prodrug Design

Scheme 2. Synthesis of Optically Active 2-Hydroxytetrahydrothienopyridine Derivatives 9a–v[a]

[a]Reagents and conditions: (a) 4-nitrobenzenesulfonyl chloride, CH_2Cl_2, Et_3N, DMAP (cat.), 0 °C; (b) $KHCO_3$, CH_3CN, rt; (c) $(R^2CO)_2O$, CH_3CN, Et_3N, 0 °C to rt, or R^2COCl, THF, Et_3N or NaH. For R^1 and R^2, see Table 1.
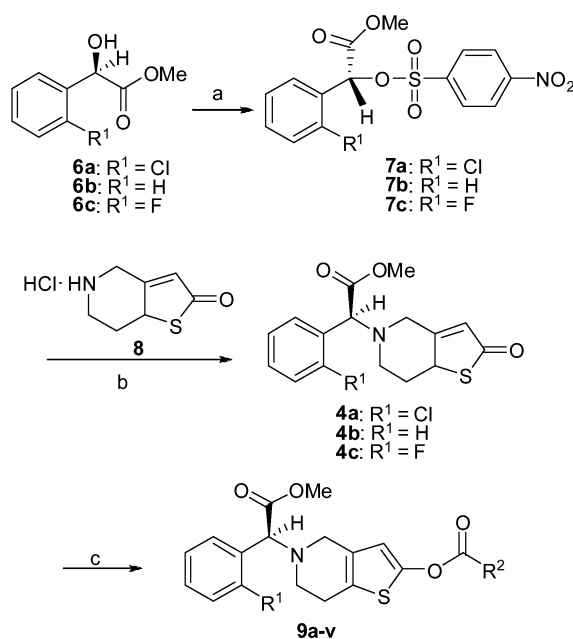
the clinical management of CR. Novel antiplatelet agents with fast onset of action, less interindividual variability, and low risk of bleeding are urgently needed.

Given the fact that clopidogrel is among the most widely prescribed drugs worldwide and its long-term safety profile has been established after 14 years of clinical use, new drug discovery based on this old drug would be highly desirable. As Nobel laureate James Black said, "The most fruitful basis for the discovery of a new drug is to start with an old drug." We envisioned that, like the metabolic pattern of prasugrel, ester prodrugs **9** might be readily converted to clopidogrel thiolactone (**4a**) by esterase-mediated hydrolysis and subsequently to clopidogrel AM (**5**) through only one CYP-dependent step (Scheme 1). In this regard, **9** could be ideal drug candidates for overcoming CR without increasing the risk of bleeding and other side effects associated with the new antiplatelet agents. Herein, we report the identification of a series of 2-hydroxytetrahydrothienopyridine derivatives as novel antiplatelet agents. We also describe the detailed structure–activity relationships (SARs) of this series of compounds. Preliminary efficacy, toxicity, and pharmacokinetic studies led to the discovery of vicagrel (**9a**) as a highly potent and orally bioavailable antiplatelet agent.

■ RESULTS AND DISCUSSION

Chemistry. Optically pure (R)-methyl 2-(2-substitued-phenyl)-2-[(4-nitrobenzenesulfonyl)oxy]acetates **7a–c** were prepared via reaction of mandelic acid and 2-substituted mandelic acids with 4-nitrobenzenesulfonyl chloride in the presence of triethylamine at low temperature (Scheme 2). N-Alkylation of 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride (**8**) with **7a–c** in the presence of potassium bicarbonate afforded thiolactones **4a–c**. Reaction of **4a–c** with acyl anhydrides, acyl chlorides, chlorocarbonic acid esters, or N-substituted carbamic chlorides in the presence of triethylamine or sodium hydride gave optically active 2-hydroxytetrahydrothienopyridine derivatives **9a–v** with (S)-configuration. The enantiomer of **9a** ((R)-**9a**) and the racemic mixture of **9a** ((R,S)-**9a**) were prepared starting from optically pure (S)-

methyl 2-(2-chlorophenyl)-2-[(4-nitrobenzenesulfonyl)oxy]-acetate and racemic methyl 2-(2-chlorophenyl)-2-[(4-nitrobenzenesulfonyl)oxy]acetate, respectively. The optical purity (% ee) of compounds **9a–v** and (R)-**9a** was determined by chiral HPLC.

Inhibition of ADP-Induced Platelet Aggregation in Rats and SAR Analysis. Twenty-five 2-hydroxytetrahydrothienopyridine derivatives were evaluated for their inhibitory effect on ADP-induced platelet aggregation in rats at dose of 3 mg/kg. Clopidogrel (**1**) and prasugrel (**2**) were used as positive controls. ADP-induced platelet aggregation was determined by Born's method.[8] The assay results are summarized in Table 1. In our preliminary tests, although clopidogrel showed strong potency at a dose of 10 mg/kg (data not shown), it was almost inactive at a dose of 3 mg/kg. Prasugrel was the most potent one among this set of test compounds. Compound **9a** exhibited very strong inhibitory effect on platelet aggregation and was only slightly less potent than prasugrel. 2-Chloro substitution seemed to be critical for the potency (e.g., **9a–c,l–o**), since both 2-hydrogen and 2-fluoro substitution resulted in significant loss of potency (e.g., **9u**, **9v**). 2-Hydroxy ester moiety of the tetrahydrothienopyridine ring also had a significant impact on potency. Interestingly, when the size of 2-hydroxy alkyl esters increased, the potency decreased accordingly (e.g., potency in the order **9a** > **9b** > **9c** > **9d** > **9e**), suggesting that the hindrance at the 2-ester groups might reduce their hydrolysis rates and thus result in lower concentrations of clopidogrel AM. The same tendency was also observed with carbonic acid esters (e.g., potency, **9m** > **9n** > **9p** > **9q**). It was notable that the potency of carbonic acid ester **9m** was similar to that of alkyl ester **9a**, indicating that carbonic acid esters could also be converted to clopidogrel AM as well as alkyl esters. Not surprisingly, clopidogrel thiolactone (**4a**) was a potent antiplatelet agent, albeit less potent than its

Table 1. Inhibitory Effect of 2-Hydroxytetrahydrothienopyridine Derivatives on ADP-Induced Platelet Aggregation in Rats at a Dose of 3 mg/kg[a]
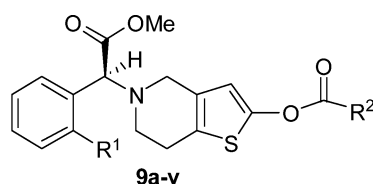

compd	R[1]	R[2]	% ee[d]	platelet aggregation (%)
1[b]			99.0	73.7 ± 5.2
2[c]				32.1 ± 9.3**
4a			98.1	48.6 ± 14.6**
9a	Cl	methyl	99.1	34.6 ± 13.5**
9b	Cl	ethyl	96.5	46.7 ± 15.4**
9c	Cl	n-propyl	96.3	52.8 ± 7.7**
9d	Cl	tert-butyl	99.1	63.4 ± 16.2*
9e	Cl	tert-amyl	99.5	70.0 ± 23.0
9f	Cl	phenyl	93.5	60.1 ± 11.9**
9g	Cl	4-NO2-phenyl	100	75.6 ± 7.0
9h	Cl	4-MeO-phenyl	96.9	82.6 ± 9.1
9i	Cl	2-AcO-phenyl	96.0	77.9 ± 4.9
9j	Cl	benzyl	93.5	53.8 ± 10.4**
9k	Cl	styryl	98.7	84.7 ± 8.7
9l	Cl	pyridine-3-yl	97.7	45.1 ± 9.0**
9m	Cl	methoxy	97.0	38.6 ± 9.1**
9n	Cl	ethoxy	97.3	53.0 ± 11.6**
9o	Cl	isopropoxy	97.5	50.2 ± 9.3**
9p	Cl	isobutoxy	95.5	65.6 ± 23.2
9q	Cl	benzyloxy	93.7	72.2 ± 10.5
9r	Cl	phenoxymethyl	89.0	75.4 ± 4.5
9s	Cl	dimethylamino	95.5	67.7 ± 17.7
9t	Cl	pyrrolidine-1-yl	95.7	80.9 ± 9.2
9u	H	methyl	72.0	81.1 ± 6.0
9v	F	methyl	86.9	62.9 ± 12.8*
(R)-9a			98.7	68.4 ± 19.6
(R,S)-9a				63.7 ± 8.0*
vehicle				75.7 ± 7.7

[a]Assay details are described in Experimental Section. Aggregation data refer to ex vivo measurements 2 h after oral administration. (∗) $P < 0.05$. (∗∗) $P < 0.01$ vs vehicle. Data are the mean ± SD, $n = 10$. [b]Clopidogrel hydrogen sulfate was used as a positive control. [c]Prasugrel free base was used as a positive control. [d]ee refers to enantiomeric excess.

acetic ester 9a. Aromatic acid esters (e.g., 9f, 9g, 9h, 9i) were not as potent as alkyl esters, except for nicotinate ester 9l which exhibited strong potency. Substituted carbamate esters were inactive in this assay (e.g., 9s, 9t). Preliminary tests had shown that 9a seemed to be the most promising drug candidate among this series of compounds, and thus, its enantiomer ((R)-9a) and racemic mixture ((R,S)-9a) were synthesized and biologically evaluated in order to investigate the effect of configuration on potency. In contrast to 9a, (R)-9a was almost inactive, while (R,S)-9a had very weak activity. On the basis of the above results, 9a was selected for further in vitro and in vivo metabolism studies.

In Vitro Metabolic Activation Studies on Clopidogrel and 9a in Rat Liver Microsomes (RLMs). Clopidogrel (1) could be metabolically activated to form its active metabolite (AM, 5) via the thiolactone intermediate (4a) under in vitro incubation conditions with liver microsomes[9] or cDNA-expressed P450 isozymes[10] (Figure 2). The active metabolite (5) is chemically labile probably because of the reactive thiol function. Although it was reported that 5 might be stable at 4 °C for 24 h in quenched incubation mixtures,[10] for the

convenience of LC−MS/MS-based qualitative and quantitative analyses as well as further purification for NMR studies, 5 was often derivatized in the incubation mixture with a variety of reagents that were reactive toward the thiol function including glutathione (GSH), acrylonitrile, 3'-methoxyphenacyl bromide, N-ethylmaleimide, and dimedone.[10,11] In this study, glutathione was used to derivatize 5 to form the active metabolite−glutathione disulfide adducts (AMGS, Figure 2). To test the metabolic activation of 9a and potential formation of the active metabolite (5), rat liver microsomal incubation was conducted for 9a in parallel with clopidogrel in the presence of NADPH and glutathione. After quenching and centrifugation, the supernatant was analyzed by LC−MS/MS. Incubation of clopidogrel with rat liver microsomes in the presence of GSH leads to NADPH-dependent formation of metabolites (Figure 3A) that exhibited a molecular ion at m/z of 661 and a product ion spectrum that is in complete agreement with the formation of the clopidogrel AMGS disulfide adducts. LC−MS/MS analysis of the supernatant resulting from incubation of 9a with rat liver microsomes in the presence of GSH reveals NADPH-dependent formation of glutathione adducts at the same LC
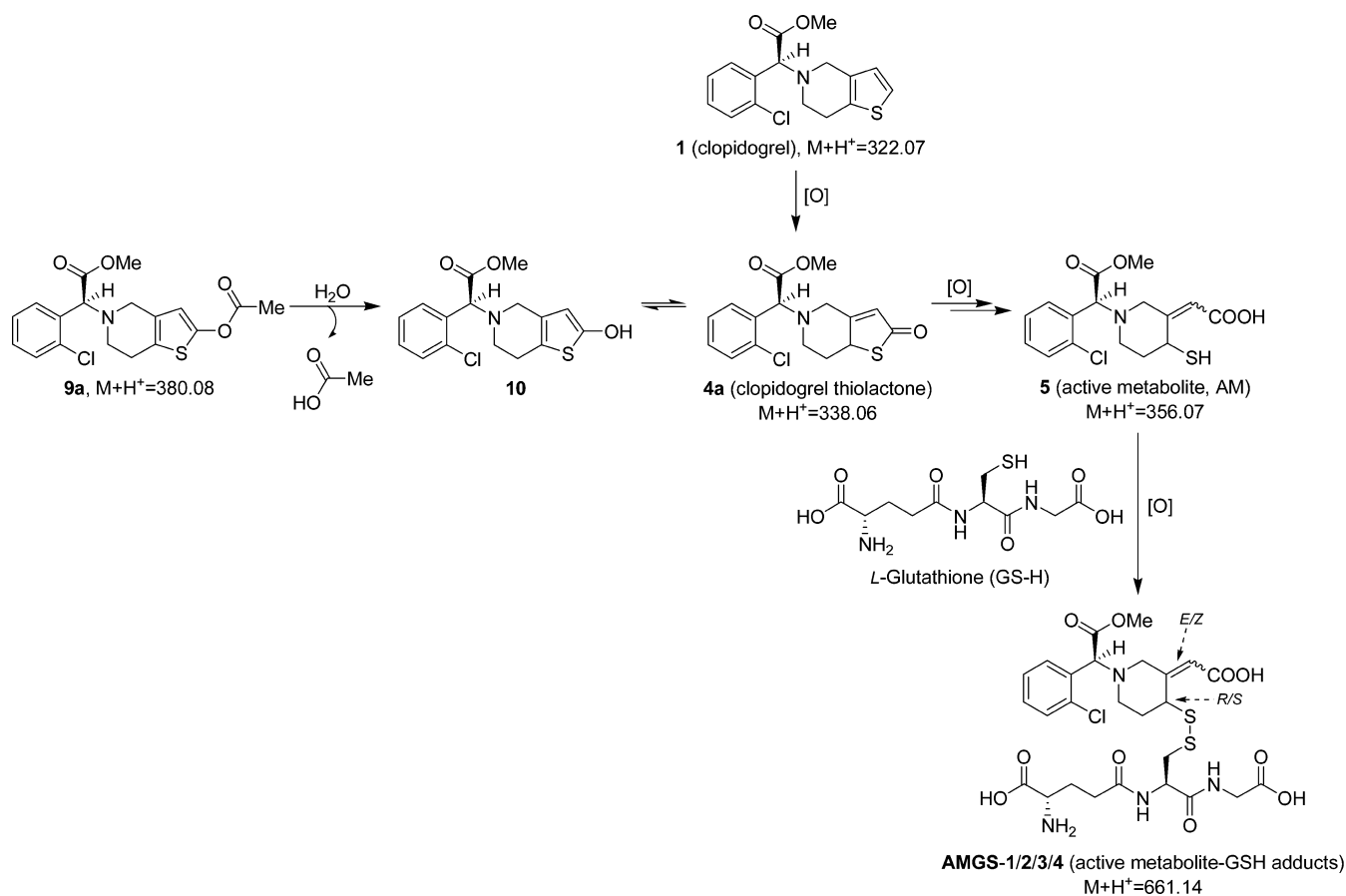
Figure 2. Proposed pathways of active metabolite and active metabolite−glutathione adduct formation via in vitro metabolic activation of clopidogrel and **9a** upon incubation with RLM in the presence of GSH and NADPH.
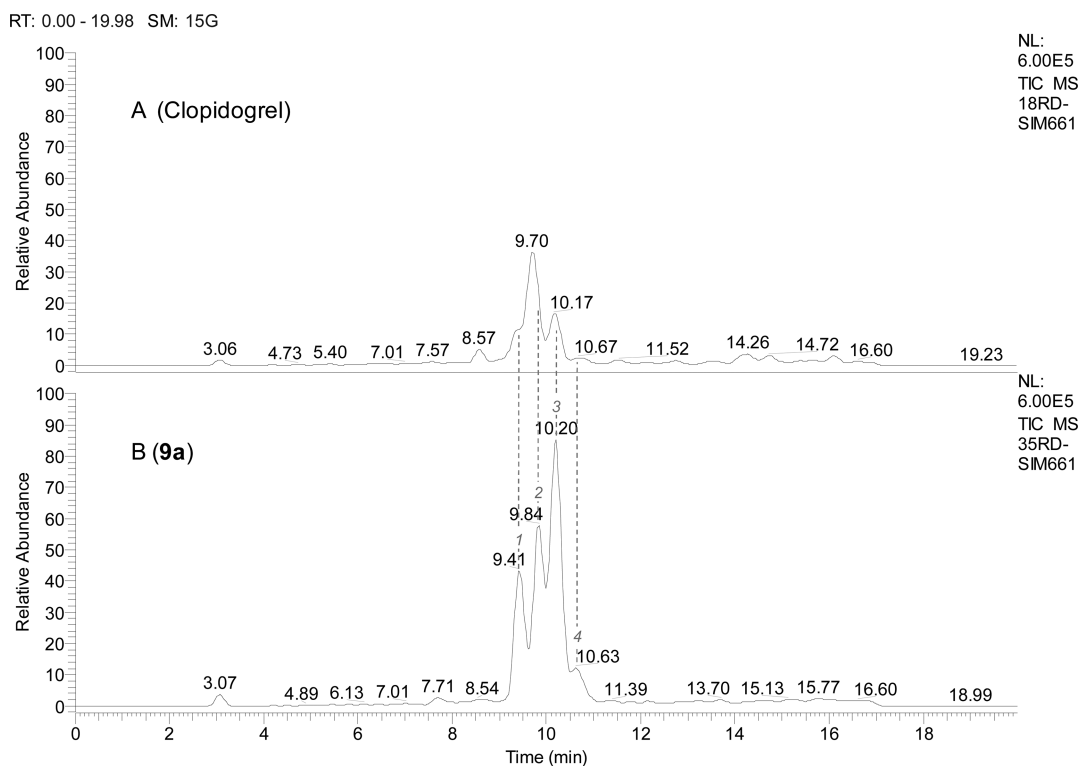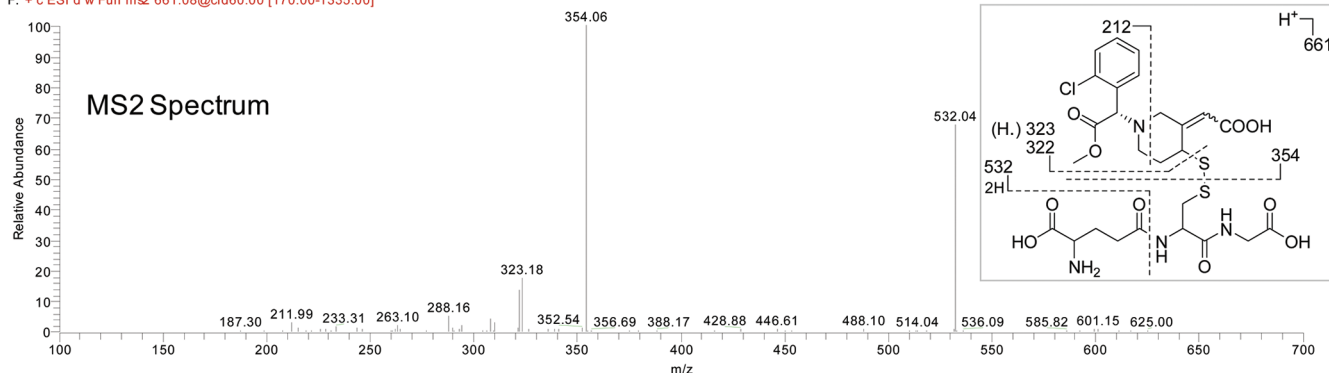

Figure 3. LC−MS-selective ion monitoring chromatogram (SIM, M + H$^+$ = 661) of active metabolite−glutathione (AMGS−**1/2/3/4**) adducts in RLM incubation of clopidogrel (A) or **9a** (B) for 30 min.
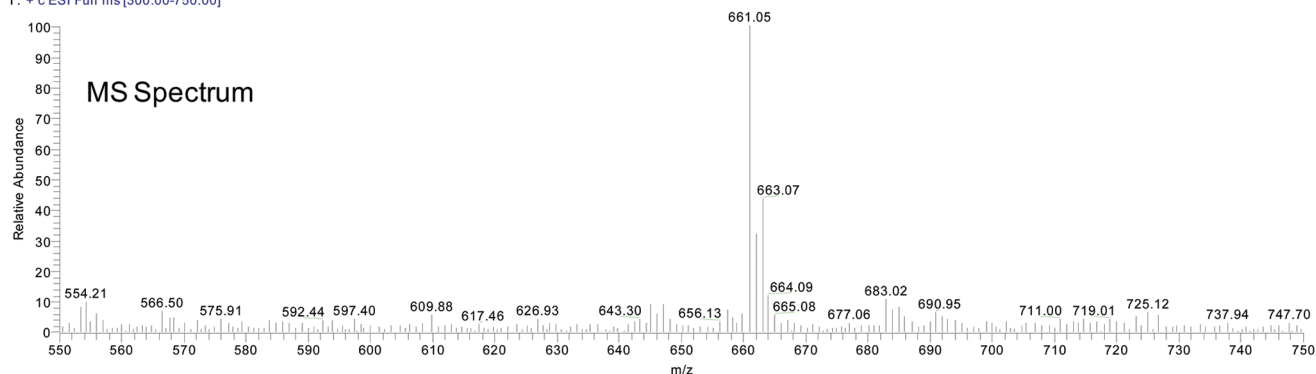
Figure 4. Averaged molecular ion (MS, top) spectrum and product ion (MS2, bottom) spectrum of the glutathione (AMGS) adducts detected in rat liver microsomal incubation of **9a** in the presence of NADPH and glutathione. The inset is proposed fragmentation pathways of the molecular ion $[M + H]^+$ of AMGS at m/z of 661.

retention time as the AMGS adducts formed in clopidogrel incubation (Figure 3, B). The molecular ion (MS) spectrum (Figure 4, top) and product ion (MS2) spectrum (Figure 4, bottom) of the glutathione adducts detected in rat liver microsomal incubation of **9a** were almost identical to those of the AMGS adducts in clopidogrel incubation (data not shown). The MS spectra of the glutathione adducts show the typical isotopic pattern that is in accordance with the monochloro-containing nature of the AMGS adducts. Fragmentation analysis (Figure 4, inserted) based on the exclusive product ions resulting from collision-induced dissociation (CID) of the molecular ion $[M + H]^+$ of m/z 661 (MS2 spectrum) confirms the formation of the AMGS adducts with the presence of both moieties of **9a** and glutathione as well as the disulfide bond formation. The metabolic activation of **9a** or clopidogrel confers on the structure of the active metabolite two additional stereochemical sites: one chiral center adjacent to the thio function and one geometric center of the ethylenic double bond, which provide the basis for the formation of four diastereomers.[12] Chromatographic separation resolved the four diastereomeric AMGS adducts formed from **9a** into four peaks with retention times of 9.41 min (AMGS−**1**), 9.84 min (AMGS−**2**), 10.02 min (AMGS−**3**), and 10.63 min (AMGS−**4**) (Figure 3B). On the basis of the LC−MS/MS studies of in vitro rat liver microsomal incubation of **9a** (see Supporting Information) paralleled to clopidogrel, the metabolic activation pathways of **9a** are proposed in Figure 2. As shown in the first step, the acetate ester function of **9a** undergoes hydrolysis probably by esterases presented in rat liver microsomes and gets deacetylated. The formed 2-hydroxytetrahydrothienopyr-

idine (**10**) is a tautomer of thiolactone **4a**, which is the metabolic intermediate resulting from the first oxidative activation of clopidogrel (**1**). The metabolic activation pathways of clopidogrel and **9a** converge after the first steps through this 2-hydroxytetrahydrothienopyridine−thiolactone tautomerization and share the subsequent pathways including the second step of NAPDH-dependent oxidative ring-opening of **4a** that eventually lead to active metabolite formation (Figure 2).

Pharmacokinetic Parameters of Clopidogrel Thiolactone after Oral Administration of Clopidogrel or 9a to Rats. The dose of clopidogrel or **9a** at 24 μmol kg^{-1} was orally administered to SD male rats. Blood was collected at 0 h (before dosing) and 0.25, 0.5, 1, 2, 4, 6, 8, 24 h postdose. The dose of clopidogrel thiolactone at 8 μmol kg^{-1} was intravenous administration to SD male rats to determine the conversion rate or bioavailability of clopidogrel or **9a** to clopidogrel thiolactone. Blood was collected at 0 h (before dosing) and 0.083, 0.167, 0.5, 1, 2, 4, 6, 8, 24 h postdose. After sample cleanup, the plasma samples were subjected to LC−MS/MS analysis to determine the plasma concentrations of clopidogrel thiolactone (Figure 5). After oral dosing of clopidogrel, the C_{max}, T_{max}, $t_{1/2}$, and $AUC_{0−\infty}$ of clopidogrel thiolactone in plasma were 6.93 ± 3.36 μg L^{-1}, 0.583 ± 0.382 h, 2.48 ± 0.466 h, and 32.2 ± 10.9 μg·h L^{-1}, respectively. After oral dosing of **9a**, the C_{max}, T_{max}, $t_{1/2}$, and $AUC_{0−\infty}$ of clopidogrel thiolactone in plasma were 67.2 ± 42.3 μg L^{-1}, 1.17 ± 0.764 h, 2.19 ± 1.68 h, and 211 ± 119 μg·h L^{-1}, respectively. The aforementioned data proved that after oral administration, **9a** could be readily converted into clopidogrel thiolactone, and bioavailability of
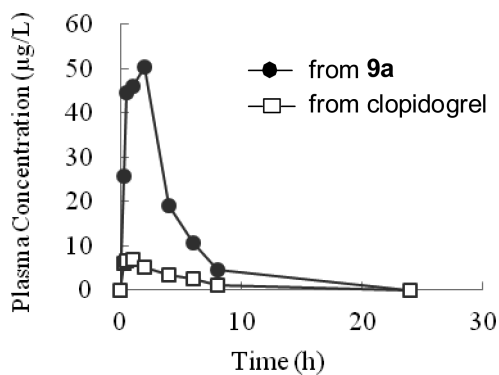
Figure 5. Plasma concentrations of clopidogrel thiolactone after oral administration of clopidogrel or **9a** to SD rats at a dose of 24 μmol kg^{-1}.

clopidogrel thiolactone generated from **9a** was 6-fold higher than that generated from clopidogrel at the same dose.

Single-Dose Toxicity of 9a. Single-dose toxicity studies on **9a** were performed in GLP laboratories. No mouse (n = 10 males, 10 females) died after receiving 5000 mg/kg **9a** by oral gavage in the 14-day observation period. Liver injury was observed, and no adverse effects were observed in other organs. After oral administration of **9a** to beagle dogs, the no-observed-adverse-effect level (NOAEL) was 300 mg/kg and the maximal tolerance dose (MTD) was higher than 2000 mg/kg. The above data indicate that **9a** has very low acute toxicity.

■ CONCLUSIONS

Although in 2010, the FDA issued a "boxed warning" of reduced effectiveness of clopidogrel in patients carrying CYP2C19 loss-of-function alleles, there is still debate on whether there is a significant association between CYP2C19 genotype and cardiovascular events in patients receiving clopidogrel.[5,13,14] Nevertheless, it seems no doubt that CYP2C19 loss-of-function alleles lead to clopidogrel resistance. In this regard, the cardiovascular risk associated with clopidogrel resistance would be definitely an unnegligible factor, especially for severe ACS patients with PCI, since platelet-mediated thrombotic events might be amplified in the presence of both plaque disruption and interventional procedures.[15] On the other hand, most of the study population involved in the large-scale clinical trials on clopidogrel were Caucasians among whom the percent of CYP2C19 poor metabolizers is only about 2%. By contrast, the percent of CYP2C19 poor metabolizers in East Asians is much higher at 15−23%.[16] That is, the meta-analysis results[13,14] based on those clinical trials may not truly reflect the risk associated with clopidogrel resistance, especially for East Asian patients. Large-scale clinical trials addressing clopidogrel resistance are warranted to define the risk of major adverse cardiovascular outcomes among CYP2C19 poor metabolizers treated with clopidogrel.

In the present study, prodrug design based on clopidogrel thiolactone metabolite was mainly aimed at overcoming clopidogrel resistance. Therefore, 25 2-hydroxytetrathie-nopyridine derivatives were synthesized and evaluated for their inhibitory effect on ADP-induced platelet aggregation in rats. The animal study results showed that some compounds (e.g., **9a**, **9b**, **9j**, **9l**, **9m**, **9n**, **9o**) exhibited potent activity as antiplatelet agents. Compound **9a** was selected for further studies, since its potency was comparable with that of prasugrel

and was much higher than that of clopidogrel. In vitro metabolism studies revealed that, like clopidogrel, **9a** could be readily converted to clopidogrel active metabolite by rat liver microsomes. Preliminary pharmacokinetic study results showed that the bioavailability of clopidogrel thiolactone generated from **9a** was 6-fold higher than that generated from clopidogrel, implying a much lower clinically effective dose and thus lower dose-related toxicity for **9a** in comparison with clopidogrel.

In summary, **9a** holds great promise as a more potent and a safer antiplatelet agent that might have the following advantages over clopidogrel: (1) no drug resistance for CYP2C19 poor metabolizers; (2) lower dose-related toxicity due to a much lower effective dose; (3) faster onset of action due to its metabolic activation mechanism. From our point of view, the bleeding risk of **9a** should be manageable, since under a proper dosing range, the efficacy of **9a** would be equal to that of clopidogrel, and thus, in theory, the bleeding risk of **9a** should not be higher than that of clopidogrel. Further preclinical trials on **9a** (vicagrel) are currently being conducted in our laboratories to prove the above predictions.

■ EXPERIMENTAL SECTION

Chemistry. Materials and General Methods. All commercially available solvents and reagents were used without further purification. Melting points were determined with a Büchi capillary apparatus and were not corrected. ^{1}H and ^{13}C NMR spectra were recorded on an ACF* 300Q Bruker or ACF* 500Q Bruker spectrometer in CDCl$_3$, with Me$_4$Si as the internal reference, or in DMSO-d_6. Low- and high-resolution mass spectra (LRMS and HRMS) were recorded in electron impact mode. Reactions were monitored by TLC on silica gel 60 F254 plates (Qingdao Ocean Chemical Company, China). Column chromatography was carried out on silica gel (200−300 mesh, Qingdao Ocean Chemical Company, China). The purity of all final compounds was determined to be ≥95% by analytical HPLC (equipment: Agilent 1100 system with a VWD G1314A UV detector; column, Chiralpak IC, 4.6 mm × 250 mm). Detailed analytical HPLC conditions for each final compound are described in the following section.

(*R*)-Methyl 2-(2-Chlorophenyl)-2-(4-nitrophenylsulfonyloxy)acetate (7a). To a stirred mixture of (*R*)-2-hydroxy-2-(2-chlorophenyl)acetate (98.4 g, 0.49 mol, 99.0% ee) and Et$_3$N (91 mL, 0.65 mol) in CH$_2$Cl$_2$ (500 mL) at 0 °C was slowly added a solution of 4-nitrobenzenesulfonyl chloride (120 g, 0.54 mol) in CH$_2$Cl$_2$ (500 mL). After being stirred for 4 h at the same temperature, the mixture was quenched with water. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was recrystallized in MeOH to afford the title compound as a light yellow solid (154.5 g, 82.0% yield), 98.1% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 50% *n*-hexane + 50% *i*-PrOH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 220 nm). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.30 (d, 2 H, *J* = 8.9 Hz), 8.07 (d, 2 H, *J* = 8.9 Hz), 7.39−7.21 (m, 4 H), 6.39 (s, 1 H), 3.57 (s, 3 H). ESI-MS *m/z* 408.0 [M + Na]$^+$.

(*S*)-Methyl 2-(2-Chlorophenyl)-2-(2-oxo-7,7a-dihydrothieno-[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate (4a). To a solution of (*R*)-methyl 2-(2-chlorophenyl)-2-(4-nitrophenylsulfonyloxy)acetate (58.1 g, 0.15 mol) in CH$_3$CN (500 mL) were added 5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one hydrochloride (32.3 g, 0.17 mol) and potassium bicarbonate (37.8 g, 0.38 mol). After being stirred at room temperature for 26 h under N$_2$ atmosphere, the mixture was filtered and the liquid was concentrated under reduced pressure. The residue was purified by column chromatography to give a yellow oil, which was recrystallized in EtOH to afford the title compound as a white solid (35.4 g, 70% yield), mp 146−148 °C, 98.1% ee (determined after conversion to **9a**). [α]$_D^{19}$ +114.0° (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.54−7.51 (m, 1 H), 7.44−

7.41 (m, 1 H), 7.32–7.26 (m, 2 H), 6.02 (s, 1 H), 4.91 (s, 1 H), 4.16 (m, 1 H), 3.92 (m, 1 H), 3.73 (s, 3 H), 3.25 (d, 1 H, J = 12.3 Hz), 3.03 (d, 1 H, J = 12.7 Hz), 2.65–2.59 (m, 1 H), 2.37–2.31 (m, 1 H), 1.93– 1.79 (m, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 198.4, 170.7, 167.1, 134.8, 132.8, 130.0, 129.7, 127.1, 126.7, 67.2, 52.2, 51.5, 51.0, 49.6, 33.8. ESI-MS m/z 338.1 [M + H]$^+$. HRMS calcd for C$_{16}$H$_{17}$NO$_3$SCl [M + H]$^+$ m/z 338.0618, found 338.0626.

(S)-Methyl 2-(2-Acetoxy-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-(2-chlorophenyl)acetate (9a). To a stirred mixture of **4a** (6.5 g, 19 mmol, 97.5% ee) and Et$_3$N (5.4 mL, 38.5 mmol) in CH$_3$CN (100 mL) at 0 °C was slowly added acetic anhydride (3.6 mL, 38.5 mmol). The mixture was stirred for 2 h at room temperature and quenched with water. Then AcOEt (100 mL) was added to the mixture and the organic layer was washed with saturated sodium bicarbonate solution, brine and dried over anhydrous sodium sulfate. The organic layer was concentrated under reduced pressure and the residue was purified by column chromatography to afford a light yellow oil, which was recrystallized in EtOH to afford the title compound as a white solid (6.8 g, 93.2% yield), mp 73–75 °C, 99.1% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 92% n-hexane + 8% THF + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{23}$ +45.00° (c 1.0, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70–7.24 (m, 4 H), 6.26 (s, 1 H), 4.92 (s, 1 H), 3.72 (s, 3 H), 3.60 (ABq, 2 H, J = 14.2 Hz), 2.90 (s, 2H), 2.79–2.65 (m, 2 H), 2.26 (s, 3 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 170.7, 167.2, 149.1, 134.2, 133.3, 129.4, 129.3, 128.9, 128.8, 126.6, 125.3, 111.5, 67.3, 51.6, 49.8, 47.6, 24.5, 20.2. ESI-MS m/z 380.0 [M + H]$^+$. HRMS calcd for C$_{18}$H$_{19}$NO$_4$SCl [M + H]$^+$ m/z 380.0723, found 380.0737.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Propionate (9b). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of propionic anydride was used in place of acetic anhydride, the title compound was obtained as a colorless oil in 66.0% yield, 96.5% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 90% n-hexane + 10% i-PrOH + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{20}$ +36.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69–7.26 (m, 4 H), 6.26 (s, 1 H), 4.91 (s, 1 H), 3.72 (s, 3 H), 3.59 (ABq, 2 H, J = 14.2 Hz), 2.88–2.87 (m, 2 H), 2.78–2.76 (m, 2 H), 2.55 (q, 2 H, J = 7.7 Hz), 1.23 (t, 3 H, J = 7.4 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 149.8, 123.7, 130.0, 129.8, 129.5, 129.1, 127.2, 125.6, 111.7, 106.2, 67.8, 52.2, 50.3, 48.2, 27.4, 25.0, 21.1, 8.8. ESI-MS m/z 394.1 [M + H]$^+$. HRMS calcd for C$_{19}$H$_{21}$NO$_4$SCl [M + H]$^+$ m/z 394.0883, found 394.0880.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Butyrate (9c). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of butyric anhydride was used in place of acetic anhydride, the title compound was obtained in 41.0% yield, 96.3% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +32.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69–7.24 (m, 4 H), 6.25 (s, 1 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.58 (ABq, 2 H, J = 14.3 Hz), 2.89–2.86 (m, 2 H), 2.78–2.76 (m, 2 H), 2.52–2.47 (m, 2 H), 1.74 (q, 2 H, J = 5.2 Hz), 1.00 (t, 3 H, J = 5.2 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 170.4, 149.7, 134.7, 133.8, 130.0, 129.8, 129.4, 129.2, 127.1, 125.7, 111.8, 67.9, 52.1, 50.3, 48.2, 35.8, 25.0, 18.2, 13.5. ESI-MS m/z 408.1 [M + H]$^+$. HRMS calcd for C$_{20}$H$_{23}$NO$_4$SCl [M + H]$^+$ m/z 408.1035, found 408.1036.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Pivalate (9d). To a solution of **4a** (337.5 mg, 1.0 mmol, 97.5% ee) in THF (15 mL) was added Et$_3$N (836 μL, 5.9 mmol). After being stirred for 10 min, the mixture was cooled to 0 °C and pivaloyl chloride (738 μL, 6.1 mmol) was added. After being stirred at 25 °C for 4 h, the mixture was poured into saturated sodium bicarbonate solution (60 mL) and extracted with EtOAc (30 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography to give the title compound in 85% yield, 99.1% ee (Chiral HPLC analytical conditions:

same as those for **9a**). $[\alpha]_D^{20}$ +38.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ7.69–7.23 (m, 4 H), 6.26 (s, 1 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.59 (ABq, 2 H, J = 14.1 Hz), 2.88–2.87 (m, 2 H), 2.79–2.77 (m, 2 H), 1.30 (s, 9 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 175.1, 171.2, 150.0, 134.6, 133.7, 129.9, 129.7, 129.3, 129.0, 127.1, 125.5, 111.3, 67.7, 52.0, 50.2, 48.1, 39.0, 26.9, 24.9. ESI-MS m/z 422.2 [M + H]$^+$. HRMS calcd for C$_{21}$H$_{25}$NO$_4$SCl [M + H]$^+$ m/z 422.1198, found 422.1193.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl 2,2-Dimethylbutanoate (9e). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of 2,2-dimethylbutanoyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a white solid in 74.9% yield, mp 98–100 °C, 99.5% ee (Chiral HPLC analytical conditions: same as those for **9a**). $[\alpha]_D^{20}$ +36.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69–7.22 (m, 4 H), 6.25 (s, 1 H), 4.90 (s, 1 H), 3.71 (s, 3 H), 3.59 (ABq, 2 H, J = 14.3 Hz), 2.88–2.87 (m, 2 H), 2.78–2.76 (m, 2 H), 1.64 (q, 2 H, J = 7.3 Hz), 1.26 (s, 6 H), 0.88 (t, 3 H, J = 7.4 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.7, 171.2, 150.0, 134.7, 133.7, 129.9, 129.7, 129.4, 129.1, 127.1, 125.5, 111.4, 67.8, 52.1, 50.3, 48.1, 43.1, 33.3, 24.9, 24.4, 9.2. ESI-MS m/z 436.2 [M + H]$^+$. HRMS calcd for C$_{22}$H$_{27}$NO$_4$SCl [M + H]$^+$, m/z 436.1352, found 436.1349.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Benzoate (9f). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of benzoic anhydride was used in place of acetic anhydride, the title compound was obtained as a white solid in 52.0% yield, mp 84–86 °C, 93.5% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +34.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.17–7.26 (m, 9 H), 6.42 (s, 1 H), 4.95 (s, 1 H), 3.73 (s, 3 H), 3.68–3.57 (m, 2 H), 2.93–2.82 (m, 4 H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 163.5, 149.9, 134.7, 133.9, 130.2, 130.0, 129.8, 129.5, 128.6, 128.5, 127.2, 125.9, 112.1, 67.8, 52.2, 50.4, 48.2, 25.0. ESI-MS m/z 442.1 [M + H]$^+$. HRMS calcd for C$_{23}$H$_{21}$NO$_4$SCl [M + H]$^+$ m/z 442.0891, found 442.0880.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl 4-Nitrobenzoate (9g). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of 4-nitrobenzoyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a yellow solid in 26.0% yield, mp 100–102 °C, 100% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 50% n-hexane + 50% i-PrOH + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{20}$ +30.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.18 (s, 4 H), 7.70–7.26 (m, 4 H), 6.47 (s, 1 H), 4.95 (s, 1 H), 3.73 (s, 3 H), 3.70–3.57 (m, 2 H), 2.95–2.91 (m, 2 H), 2.84–2.82 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.3, 161.7, 151.0, 149.2, 134.8, 133.9, 133.6, 131.3, 130.0, 129.8, 129.5, 127.2, 126.6, 123.8, 112.6, 67.8, 67.3, 52.2, 51.6, 51.1, 50.3, 49.7, 48.1, 29.7, 25.1. ESI-MS m/z 487.0 [M + H]$^+$. HRMS calcd for C$_{23}$H$_{20}$N$_2$O$_6$SCl [M + H]$^+$ m/z 487.0736, found 487.0731.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl 4-Methoxybenzoate (9h). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of 4-methoxybenzoyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a yellow oil in 30.0% yield, 96.9% ee (Chiral HPLC analytical conditions: same as those for **9g**). $[\alpha]_D^{20}$ +26.00° (c 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.09–8.06 (m, 2 H), 7.68–7.26 (m, 4 H), 6.95–6.92 (m, 2 H), 6.38 (s, 1 H), 4.92 (s, 1 H), 3.85 (s, 5 H), 3.71–3.59 (m, 5 H), 2.90–2.79 (m, 4 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 164.5, 163.1, 149.9, 134.6, 133.7, 132.7, 132.3, 129.9, 129.7, 129.4, 127.1, 125.7, 121.2, 120.6, 114.1, 113.6, 111.8, 67.8, 55.4, 52.0, 50.3, 48.1, 25.0. ESI-MS m/z 472.2 [M + H]$^+$, 494.2 [M + Na]$^+$. HRMS calcd for C$_{24}$H$_{23}$NO$_5$SCl [M + H]$^+$ m/z 472.0993, found 472.0985.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl 2-Acetoxybenzoate (9i). Following a procedure similar to that described for the preparation

of **9d** except that an equivalent amount of 2-(chlorocarbonyl)phenyl acetate was used in place of pivaloyl chloride, the title compound was obtained as a pale yellow oil in 56.0% yield, 96.0% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 85% *n*-hexane + 15% THF + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{20}$ +14.00° (*c* 0.50, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.15–7.39 (m, 4 H), 7.36–7.14 (m, 4 H), 6.37 (s, 1 H), 4.93 (s, 1 H), 3.72 (s, 3 H), 3.66–3.54 (m, 2 H), 2.93–2.90 (m, 2 H), 2.81–2.79 (m, 2 H), 2.34 (s, 3 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.17, 169.47, 161.32, 151.12, 149.32, 135.73, 134.71, 133.54, 132.12, 130.71, 129.92, 129.79, 129.45, 129.35, 127.12, 126.14, 124.09, 121.66, 118.97, 117.34, 112.61, 67.65, 52.12, 50.12, 48.04, 24.93, 20.93. ESI-MS *m/z* 500 [M + H]$^+$, 522 [M + Na]$^+$. HRMS calcd for C$_{25}$H$_{23}$NO$_6$SCl [M + H]$^+$ *m/z* 500.0931, found 500.0935.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(2-phenylacetoxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9j). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of 2-phenylacetyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a yellow oil in 46.0% yield, 93.5% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +14.00° (*c* 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.68–7.64 (m, 1 H), 7.41–7.23 (m, 8 H), 6.26 (s, 1 H), 4.89 (s, 1 H), 3.82 (s, 2 H), 3.71 (s, 3 H), 3.57 (ABq, 2 H, J = 14.3 Hz), 2.88–2.86 (m, 2 H), 2.77–2.75 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 168.3, 149.6, 134.7, 133.7, 132.7, 129.9, 129.8, 129.4, 129.2, 129.1, 128.7, 127.4, 127.1, 125.8, 111.9, 67.8, 52.1, 50.2, 48.1, 40.8, 29.6, 24.9. ESI-MS *m/z* 456.2 [M + H]$^+$, 478.2 [M + Na]$^+$. HRMS calcd for C$_{24}$H$_{23}$NO$_4$SCl [M + H]$^+$ *m/z* 456.1041, found 456.1036.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Cinnamate (9k). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of cinnamoyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a yellow solid in 34.6% yield, mp 122–124 °C, 98.7% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 90% *n*-hexane + 10% THF + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{20}$ +14.00° (*c* 0.50, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.85 (d, 1 H, J = 15.9 Hz), 7.71–7.69 (m, 1 H), 7.58–7.55 (m, 2 H), 7.42–7.39 (m, 4 H), 7.32–7.24 (m, 2 H), 6.56 (d, 1 H, J = 15.9 Hz), 6.35 (s, 1 H), 4.92 (s, 1 H), 3.72 (s, 3 H), 3.66–3.48 (m, 2 H), 2.90–2.89 (m, 2 H), 2.81–2.79 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 163.6, 147.3, 134.7, 133.9, 130.9, 129.8, 129.4, 128.9, 128.6, 128.3, 127.1, 125.8, 116.0, 111.7, 67.8, 52.1, 50.3, 48.1, 25.0. ESI-MS *m/z* 468.2 [M + H]$^+$, 490.2 [M + Na]$^+$. HRMS calcd for C$_{25}$H$_{23}$NO$_4$SCl [M + H]$^+$ *m/z* 468.1032, found 468.1036.

(S)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl Nicotinate (9l). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of nicotinoyl chloride was used in place of pivaloyl chloride, the title compound was obtained as a yellow solid in 38.8% yield, mp 92–94 °C, 97.7% ee (Chiral HPLC analytical conditions: Chiralpak IC, 4.6 mm × 250 mm, eluting with 50% *n*-hexane + 50% *i*-PrOH + 0.1% Et$_2$NH, flow rate 0.5 mL/min, oven temperature 25 °C, detection UV 254 nm). $[\alpha]_D^{20}$ +34.00° (*c* 0.50, MeOH). ^{1}H NMR (500 MHz, CDCl$_3$): δ 9.34 (m, 1 H), 8.84–8.83 (m, 1 H), 8.41–8.38 (m, 1 H), 7.69 (m, 1 H), 7.45–7.25 (m, 4 H), 6.45 (s, 1 H), 4.93 (s, 1 H), 3.71 (s, 3 H), 3.69–3.58 (m, 2 H), 2.93–2.91 (m, 2 H), 2.82–2.80 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.16, 162.17, 154.07, 151.19, 149.18, 137.54, 134.68, 133.59, 129.89, 129.78, 129.43, 129.32, 127.11, 126.33, 124.66, 123.47, 112.43, 67.73, 52.11, 50.24, 48.05, 24.93. ESI-MS *m/z* 443.1 [M + H]$^+$, 465.1 [M + Na]$^+$. HRMS calcd for C$_{22}$H$_{20}$N$_2$O$_4$SCl [M + H]$^+$ *m/z* 443.0839, found 443.0832.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(methoxycarbonyloxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9m). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of methyl carbonochloridate was

used in place of pivaloyl chloride, the title compound was obtained in 39.0% yield, 97.0% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +24.00° (*c* 0.50, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.68–7.23 (m, 4 H), 6.29 (s, 1 H), 4.90 (s, 1 H), 3.90 (s, 3 H), 3.72 (s, 3 H), 3.58 (ABq, 2 H, J = 14.2 Hz), 2.90–2.83 (m, 2 H), 2.78–2.75 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 153.3, 150.4, 134.7, 133.7, 129.9, 129.8, 129.6, 129.4, 127.1, 126.0, 112.6, 67.8, 55.8, 52.1, 50.3, 48.1, 25.1. ESI-MS *m/z* 396.1 [M + H]$^+$. HRMS calcd for C$_{18}$H$_{19}$NO$_5$SCl [M + H]$^+$ *m/z* 396.0672, found 396.0675.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(ethoxycarbonyloxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9n). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of ethyl carbonochloridate was used in place of pivaloyl chloride, the title compound was obtained as a white solid in 74.7% yield, mp 42–44 °C, 97.3% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +40.00° (*c* 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.68–7.24 (m, 4 H), 6.30 (s, 1 H), 4.90 (s, 1 H), 4.32 (q, 2 H, J = 7.1, 14.3 Hz), 3.72 (s, 3 H), 3.58 (ABq, 2 H, J = 14.2 Hz), 2.90–2.86 (m, 2 H), 2.78–2.76 (m, 2 H), 1.37 (t, 3 H, J = 7.2 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.5, 157.3, 152.9, 135.0, 134.0, 130.2, 130.1, 129.8, 129.7, 127.4, 126.2, 112.7, 68.1, 65.7, 52.4, 50.5, 48.4, 25.4, 14.4. ESI-MS *m/z* 410.1 [M + H]$^+$, 432.1 [M + Na]$^+$. HRMS calcd for C$_{19}$H$_{21}$NO$_5$SCl [M + H]$^+$ *m/z* 410.0836, found 410.0829.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(isopropoxycarbonyloxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9o). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of isopropyl carbonochloridate was used in place of pivaloyl chloride, the title compound was obtained in 94.3% yield, 97.5% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +34.00° (*c* 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.68–7.24 (m, 4 H), 6.30 (s, 1 H), 5.01–4.93 (m, 1 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.57 (ABq, 2 H, J = 14.2 Hz), 2.89–2.86 (m, 2 H), 2.78–2.77 (m, 2 H), 1.36 (d, 6 H, J = 6.2 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 152.0, 150.5, 134.7, 133.7, 130.3, 129.9, 129.8, 129.4, 127.1, 125.7, 112.2, 73.9, 67.8, 52.1, 50.3, 48.1, 25.4, 21.6. ESI-MS *m/z* 424.1 [M + H]$^+$. HRMS calcd for C$_{20}$H$_{23}$NO$_5$SCl [M + H]$^+$ *m/z* 424.0989, found 424.0985.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(isobutoxycarbonyloxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9p). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of isobutyl carbonochloridate was used in place of pivaloyl chloride, the title compound was obtained in 68.6% yield, 95.5% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +16.00° (*c* 0.50, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.42–7.26 (m, 4 H), 6.29 (s, 1 H), 4.90 (s, 1 H), 4.25 (d, 2 H, J = 6.6 Hz), 3.72 (s, 3H), 3.58 (ABq, 2 H, J = 14.2 Hz), 2.90–2.86 (m, 2 H), 2.78–2.76 (m, 2 H), 2.05–2.01 (m, 1 H), 0.98 (d, 6 H, J = 6.7 Hz). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.5, 153.0, 150.8, 135.0, 134.0, 130.2, 130.1, 129.8, 129.7, 127.4, 126.1, 112.6, 75.6, 68.1, 52.4, 50.5,, 48.4, 28.0, 25.4, 19.1. ESI-MS *m/z* 460.3 [M + Na]$^+$. HRMS calcd for C$_{21}$H$_{25}$NO$_5$SCl [M + H]$^+$ *m/z* 438.1150, found 438.1142.

(S)-Methyl 2-(2-(Benzyloxycarbonyloxy)-6,7-dihydrothieno-[3,2-c]pyridin-5(4H)-yl)-2-(2-chlorophenyl)acetate (9q). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of benzyloxy carbonochloridate was used in place of pivaloyl chloride, the title compound was obtained in 81.9% yield, 93.7% ee (Chiral HPLC analytical conditions: same as those for **9b**). $[\alpha]_D^{20}$ +12.00° (*c* 0.50, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.42–7.24 (m, 4 H), 6.30 (s, 1 H), 5.26 (s, 2 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.58 (ABq, 2 H, J = 14.3 Hz), 2.90–2.86 (m, 2 H), 2.78–2.76 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 152.6, 150.4, 134.7, 134.3, 133.7, 129.9, 129.8, 129.5, 129.4, 128.9, 128.7, 128.6, 127.1, 127.0, 126.0, 112.5, 70.9, 67.8, 52.1, 50.3, 48.1, 25.1. ESI-MS *m/z* 472.1 [M + H]$^+$, 494.1 [M + Na]$^+$. HRMS calcd for C$_{24}$H$_{23}$NO$_5$SCl [M + H]$^+$ *m/z* 472.0996, found 472.0985.

(S)-Methyl 2-(2-Chlorophenyl)-2-(2-(2-phenoxyacetoxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate (9r). Following a procedure similar to that described for the preparation of **9d** except that an equivalent amount of 2-phenoxyacetyl chloride was used in

place of pivaloyl chloride, the title compound was obtained as a yellow solid in 36.0% yield, mp 104−106 °C, 89.0% ee (Chiral HPLC analytical conditions: same as those for **9b**), $[\alpha]_D^{20}$ +32.00° (*c* 0.50, MeOH). ^{1}H NMR (300 MHz,CDCl$_3$): δ 7.68−7.65 (m, 1 H), 7.42−7.24 (m, 5 H), 7.04−6.99 (m, 1 H), 6.95−6.92 (m, 2 H), 6.32 (s, 1 H), 4.90 (s, 1 H), 4.82 (s, 2 H), 3.71 (s, 3 H), 3.59 (ABq, 2 H, *J* = 14.3 Hz), 2.90−2.86 (m, 2 H), 2.78−2.77 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.1, 165.8, 157.5, 148.7, 134.7, 133.5, 129.9, 129.8, 129.6, 129.4, 129.2, 127.1, 126.1, 122.1, 114.8, 112.4, 67.7, 65.1, 52.1, 50.2, 48.0, 24.9. ESI-MS *m/z* 472.2 [M + H]$^+$, 494.2 [M + Na]$^+$. HRMS calcd for C$_{24}$H$_{23}$NO$_5$SCl [M + H]$^+$ *m/z* 472.0993, found 472.0985.

(*S*)-Methyl 2-(2-Chlorophenyl)-2-(2-(dimethylcarbamoyloxy)-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetate (9s). To a solution of **4a** (337.5 mg, 1.0 mmol, 97.5% ee) in DMF (15 mL) was added NaH (44 mg, 1.1 mmol, 60% dispersion in mineral oil) at 0 °C. After the mixture was stirred at the same temperature for 0.5 h, dimethylcarbamic chloride (386.5 μL, 4.2 mmol) was added to the mixture and stirred at 25 °C for 2 h. Then the mixture was poured into 60 mL of 10% KH$_2$PO$_4$ aqueous solution and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography to afford a yellow oil, which was recrystallized in EtOH to give the title compound as a white solid (183.6 mg, 45% yield), mp 96−98 °C, 95.5% ee (Chiral HPLC analytical conditions: same as those for **9l**). $[\alpha]_D^{20}$ +34.00° (*c* 0.50, MeOH). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.69−7.26 (m, 4 H), 6.19 (s, 1 H), 4.91 (s, 1 H), 3.72 (s, 3 H), 3.68−3.56 (m, 2 H), 3.03 (s, 3 H), 2.99 (s, 3 H), 2.89−2.85 (m, 2 H), 2.76−2.73 (m, 2 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.2, 151.0, 134.6, 134.3, 133.8, 130.5, 129.9, 129.7, 129.3, 129.0, 127.1, 125.3, 111.4, 67.8, 52.1, 50.4, 48.1, 36.8, 36.3, 25.0. ESI-MS *m/z* 409.2 [M + H]$^+$. HRMS calcd for C$_{19}$H$_{22}$N$_2$O$_4$SCl [M + H]$^+$ *m/z* 409.0992, found 409.0989.

(*S*)-5-(1-(2-Chlorophenyl)-2-methoxy-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl Pyrrolidine-1-carboxylate (9t). Following a procedure similar to that described for the preparation of **9s** except that an equivalent amount of pyrrolidine-1-carbonyl chloride was used in place of dimethylcarbamic chloride, the title compound was obtained as a white oil in 46.0% yield, 95.7% ee (Chiral HPLC analytical conditions: same as those for **9g**). $[\alpha]_D^{22}$ +19.00° (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.40−7.22 (m, 4 H), 6.20 (s, 1 H), 4.89 (s, 1 H), 3.76−3.61 (m, 4 H), 3.54−3.44 (m, 5 H), 2.89−2.77 (m, 2 H), 2.75−2.62 (m, 2 H), 2.04−1.87 (m, 4 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.3, 151.8, 151.0, 134.7, 133.9, 130.0, 129.7, 129.3, 129.1, 128.6, 127.1, 125.2, 111.5, 67.9, 52.1, 50.4, 48.2, 46.6, 46.3, 29.7, 25.7, 25.1, 24.9. ESI-MS *m/z* 435.1 [M + H]$^+$. HRMS calcd for C$_{21}$H$_{24}$N$_2$O$_4$SCl [M + H]$^+$ *m/z* 435.1145, found 435.1148.

(*S*)-Methyl 2-(2-Acetoxy-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-phenylacetate (9u). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of (*S*)-methyl 2-(2-oxo-7,7a-dihydrothieno[3,2-*c*]pyridin-5-(2*H*,4*H*,6*H*)-yl)-2-phenylacetate (**4b**) was used in place of **4a**, the title compound was obtained as a colorless oil in 93.0% yield, 72.0% ee (Chiral HPLC analytical conditions: same as those for **9a**). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69−7.25 (m, 5 H), 6.25 (s, 1 H), 4.32 (s, 1 H), 3.72 (s, 3 H), 3.54 (s, 2 H), 2.91−2.47 (m, 4 H), 2.26 (s, 3 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 167.7, 128.9, 128.7, 111.9, 72.5, 52.2, 50.3, 48.1, 24.4, 20.7. ESI-MS *m/z* 346.1 [M + H]$^+$. HRMS calcd for C$_{18}$H$_{20}$NO$_4$S [M + H]$^+$ *m/z* 346.1113, found 346.1125.

(*S*)-Methyl 2-(2-Acetoxy-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-(2-fluorophenyl)acetate (9v). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of (*S*)-methyl 2-(2-fluorophenyl)-2-(2-oxo-7,7a-dihydrothieno[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate (**4c**) was used in place of **4a**, the title compound was obtained in 86.0% yield, 86.9% ee (Chiral HPLC analytical conditions: same as those for **9a**). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.59−7.07 (m, 4 H), 6.27 (s, 1 H), 4.80 (s, 1 H), 3.74 (s, 3 H), 3.64 (s, 2 H), 2.97−2.47 (m, 4 H), 2.26 (s, 3 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 171.0, 167.7, 162.6, 159.3, 149.6, 130.3, 130.2, 130.1, 130.0, 129.0, 125.6, 124.5, 124.4,

115.8, 115.5, 111.9, 64.3, 52.2, 50.0, 48.0, 24.9, 20.7. ESI-MS *m/z* 364.1 [M + H]$^+$. HRMS calcd for C$_{18}$H$_{19}$NO$_4$SF [M + H]$^+$ *m/z* 364.1019, found 364.1029.

(*R*)-Methyl 2-(2-Acetoxy-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-(2-chlorophenyl)acetate ((*R*)-9a). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of (*R*)-methyl-2-(2-chlorophenyl)-2-(2-oxo-7,7a-dihydrothieno[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate was used in place of **4a**, the title compound was obtained in 93.1% yield, 98.7% ee (Chiral HPLC analytical conditions: same as those for **9a**). $[\alpha]_D^{23}$ −44.00° (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69−7.23 (m, 4 H), 6.25 (s, 1 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.58 (ABq, 2 H, *J* = 14.2 Hz), 2.89−2.86 (m, 2 H), 2.78−2.76 (m, 2 H), 2.26 (s, 3 H). ESI-MS *m/z* 380.0 [M + H]$^+$.

Methyl 2-(2-Acetoxy-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-(2-chlorophenyl)acetate ((*R,S*)-9a). Following a procedure similar to that described for the preparation of **9a** except that an equivalent amount of (*R,S*)-methyl-2-(2-chlorophenyl)-2-(2-oxo-7,7a-dihydrothieno[3,2-*c*]pyridin-5(2*H*,4*H*,6*H*)-yl)acetate was used in place of **4a**, the title compound was obtained in 92.1% yield. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.25−7.68 (m, 4 H), 6.26 (s, 1 H), 4.90 (s, 1 H), 3.72 (s, 3 H), 3.58 (ABq, 2 H, *J* = 14.2 Hz), 2.89−2.78 (m, 2 H), 2.78−2.76 (m, 2 H), 2.26 (s, 3 H). ESI-MS *m/z* 380.0 [M + H]$^+$.

Inhibition of ADP-Induced Platelet Aggregation in Rats. Male Wistar rats weighing 200−250 g were used (purchased from the Animal Experimental Center, Shandong University, Shandong, China) and subject to a 12 h/12 h light/dark cycle in an acclimatized room, receiving water and regular food for rodents ad libitum. The animals were divided into 28 groups of 10 animals each. Compounds were orally administered at a dose of 3 mg/kg or CMC as a vehicle. Two hours after administration, the animals were anesthetized with pentobarbital sodium, a surgical incision through the abdominal wall was made, and blood samples were collected from the abdominal aorta with Plus blood collection tubes (Becton, Dickinson and Company, U.K.) for platelet aggregation tests according to Born's method.[8] ADP (1.2 μM, Sigma Chemical Co.) was used as agonists for platelet aggregation. Platelet aggregation was measured using an aggregometer (560 Ca, Chrono-Log Co., U.S.).

Production of the Active Metabolite (5) from in Vitro Metabolic Activation of 9a or Clopidogrel in Rat Liver Microsomal Incubation. *Materials.* Molecular-biology-grade potassium phosphate monobasic, potassium hydroxide, trichloroacetic acid, ʟ-glutathione, and β-nicotinamide adenine dinucleotide 2′-phosphate (reduced, NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). Pooled Sprague−Dawley rat liver microsomes (RLM, protein concentration, 20 mg/mL) were purchased from Research Institute for Liver Diseases (RILD, Shanghai, China).

Protocol. Test compound **9a** and clopidogrel were prepared as 10 mM DMSO stock solutions. Each in vitro incubation mixture contains 1.0 mg/mL rat liver microsomes, 1.0 mM NADPH, 20 μM **9a** or clopidogrel, and 10 mM glutathione in a final volume of 200 μL of potassium phosphate (100 mM) buffer (pH 7.4). Control samples were made by replacing NADPH with potassium phosphate buffer. All the samples were incubated in a 37 °C shaking water bath for 30 min. Each incubation mixture was quenched with 30 μL of ice-cold trichloroacetic acid solution (10%, w/v) and kept on ice for 5 min before being centrifuged at 12 000 rpm for 10 min on a 5810R centrifuge (Eppendorf AG, Hamburg, Germany) to fully pelletize the precipitated proteins. The supernatant was injected into the LC−MS/MS instrument to determine the formation of active metabolite (**5**) production in the form of glutathione adducts.

LC−MS/MS Analysis of Active Metabolite (5) Formation. *Materials.* Chromatography-grade H$_2$O, MeOH, and formic acid were all purchased from Sigma-Aldrich (St. Louis, MO).

Instrumentation and Conditions. Chromatographic analysis of the supernatant resulting from the above incubation was conducted on a Surveyor HPLC system consisting of an autosampler, a MS pump, and a photodiode array detector (Thermo Fisher Scientific, Waltham, MA) using a Zorbax SB-phenyl column (4.6 mm × 75 mm, 3.5 μm, Agilent

Technologies, Santa Clara, CA) at room temperature. The volume of each injection was 20 μL. The mobile phase consisted of H_2O (solvent A, containing 0.1% formic acid) and MeOH (solvent B, containing 0.1% formic acid) and was delivered at 750 μL/min. The initial composition of solvent B was maintained at 10% for 2 min and then increased in a linear manner to 50% at 7 min and 90% at 17 min. It was then maintained at 90% solvent B for 1 min and finally decreased to 10% at 19 min. The column was allowed to equilibrate at 10% solvent B for 1 min before the ending of the 20 min gradient elution program for next injection. The scan range of the photodiode array detector was set to 220−400 nm. Mass spectrometry (MS) analysis was performed on a Thermo LCQ ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA), which was interfaced to the above HPLC system. The HPLC eluate was split after the photodiode array detector, and 10% (75 μL/min) eluate was injected onto the mass spectrometer. MS analysis was conducted using a standard electrospray ionization (ESI) source operating in positive ionization mode. Source operating conditions were 4.5 kV spray voltage, 225 °C heated capillary temperature, 20 V capillary voltage, and sheath gas flow at 40 (arbitrary unit), respectively. The MS experiment parameters including the nitrogen gas flow rate, capillary voltage, and the tube lens voltages were all tuned and optimized to give maximum detection sensitivities using a clopidogrel standard solution (10 μM in MeOH/H_2O, 1/1, v/v). The MS full scans were monitored over a mass range of m/z 300−700. Product ion (MS2) scans were generated via collision-induced dissociation (CID) with helium using normalized collision energy of 60% and a precursor ion isolation width of m/z 2.0. Data were centroid and processed in Qual Browser (Thermo Fisher Scientific). The active metabolite (5) formed via metabolic activation of 9a or clopidogrel was detected and analyzed in its more stable glutathione-derivatized form of active metabolite−glutathione adducts (AMGS−1/2/3/4). Fragmentations were proposed based on plausible protonation sites, subsequent isomerization, and even electron species, as well as bond saturation. Product ion spectra comparison between the parent and AMGS adducts further aided in the confirmation of active metabolite structure.

Pharmacokinetic Studies. Sprague−Dawley male rats (SPF grade) were used to determine oral bioavailability and PK parameters of the clopidogrel and 9a following oral and intravenous administration. In this study, clopidogrel thiolactone (4a) was measured by LC/MS/MS analysis to determine the PK parameters for both clopidogrel and 9a. PK parameters were calculated using a noncompartmental model. Solutions of clopidogrel and 9a in N,N-dimethylacetamide (DMA)/polyethylene glycol 400 (PEG 400) (v/v 5:95) at 1.26 and 1.14 mg/mL were prepared for oral dosing. A solution of clopidogrel thiolactone in DMA/PEG 400/saline (v:v:v 5:15:80) at 0.54 mg/mL was prepared for intravenous administration to determine the oral bioavailability of both clopidogrel and 9a to be converted to clopidogrel thiolactone. The solution formulations of clopidogrel thiolactone along with clopidogrel and 9a were administered separately by bolus injection into a tail vein and by oral gavage. Blood samples (about 0.20 mL) were collected from the retro-orbital plexus from three animals in each treatment group at 0 (predose), 0.083, 0.167. 0.5, 1, 2, 4, 6, 8, and 24 h for iv and 0 (predose), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h for po. Calibration standards with clopidogrel thiolactone concentrations from 0.5 to 1000 ng/mL were prepared by serial dilution in pretreated naive rat plasma. A portion (25 μL) of each calibration standard and unknown study sample was mixed with 25 μL of acetonitrile containing the internal standard followed by addition of 200 μL of MTBE into each sample. All samples were vortex-mixed for 3 min. The mixture was then centrifuged at 15700g at 4 °C for 3 min. The supernatants containing the organic component for each sample were used for analysis. The lower limit of quantitation was 0.5 ng/mL.

ASSOCIATED CONTENT

Ⓢ Supporting Information

LC−MS/MS study report on in vitro rat liver microsomal incubation of 9a; ^{1}H and ^{13}C NMR spectra for compounds 9a− v; chiral HPLC chromatograms for 9a, (R)-9a, and (R,S)-9a. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP, adenosine diphosphate; CYP, cytochrome P450; AM, active metabolite; PM, poor metabolizer; IM, intermediate metabolizer; CR, clopidogrel resistance; RLM, rat liver microsome; GSH, glutathione; AMGS, active metabolite−glutathione disulfide

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