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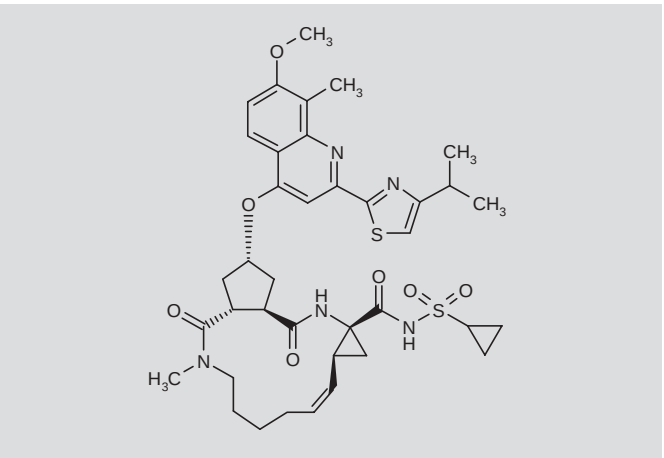
TMC-435350

HCV NS3/4A Protease Inhibitor
Anti-Hepatitis C Virus Drug

HCV-PI
TMC-00435350
TMC-435

N-[(2*R*,3a*R*,10*Z*,11a*S*,12a*R*,14a*R*)-2-[2-(4-Isopropylthiazol-2-yl)-7-methoxy-8-methylquinolin-4-yloxy]-5-methyl-4,14-dioxo-1,2,3,3a,4,5,6,7,8,9,11a,12,12a,13,14,14a-hexadecahydrocyclopenta[c]cyclopropa[g][1,6]diazacyclotetradecin-12a-ylcarbonyl]cyclopropanesulfonamide

InChI=1/C38H47N5O7S2/c1-21(2)30-20-51-35(40-30)29-18-32(26-13-14-31(49-5)22(3)33(26)39-29)50-24-16-27-28(17-24)36(45)43(4)15-9-7-6-8-10-23-19-38(23,41-34(27)44)37(46)42-52(47,48)25-11-12-25/h8,10,13-14,18,20-21,23-25,27-28H,6-7,9,11-12,15-17,19H2,1-5H3,(H,41,44)(H,42,46)/b10-8-/t23-,24-,27-,28-,38-/m1/s1



$C_{38}H_{47}N_5O_7S_2$
Mol wt: 749.939
CAS: 923604-59-5
EN: 445010

ABSTRACT

Hepatitis C is a viral infection of the liver and a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Despite programs to control the spread of the hepatitis C virus (HCV), 3-4 million people are still newly infected each year. Tibotec and Medivir are currently developing TMC-435350 (TMC-435), an inhibitor of NS3/4A protease, which plays an important role in HCV replication. Data from phase I and II clinical trials of TMC-435350 to date have shown that this agent is well tolerated as a once-daily oral therapy and provides potent antiviral activity in HCV genotype 1-infected subjects, with restoration of liver enzymes and no evidence of viral breakthrough.

SYNTHESIS

TMC-435350 can be synthesized as follows.

Ring closing metathesis of intermediate (I) by means of Hoveyda–Grubbs first-generation catalyst in 1,2-dichloroethane at 75 °C furnishes macrocycle (II), which is then treated with LiOH in THF/MeOH/H_2O to give carboxylic acid (III). Finally, activation of acid (III) by treatment with 1,1'-carbonyldiimidazole (CDI) in refluxing THF affords the oxazolidinedione derivative (IV), which is readily reacted with cyclopropylsulfonamide (V) in the presence of DBU in THF at 50 °C (1-3). Scheme 1.

Intermediate (I) can be prepared from lactone acid (VI) by two related pathways:

Lactone acid (VI) is amidated with *N*-methylhex-5-enamine (VII) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and DIEA in DMF at 0 °C. The resulting lactone amide (VIII) is opened with LiOH in THF/methanol/H_2O and the intermediate carboxylic acid is then condensed with the hydrochloride of amine (IX) by means of DIAD and HATU in DMF, producing hydroxyamide (X) (1). Finally, alcohol (X) and quinolinol derivative (XI) are coupled under Mitsunobu conditions (PPh_3, DIAD) in THF (1-3). Scheme 2.

Alternatively, lactone acid (VI) is esterified with di-*tert*-butyl dicarbonate (Boc_2O) in the presence of DMAP in CH_2Cl_2, providing the *tert*-butyl-protected lactone (XII), which is then opened by treatment with LiOH in dioxane/H_2O at 0 °C. Amidation of the in situ-generated acid with the vinylcyclopropyl amino acid ester (IX) (in its hydrochloride form) in DMF at 0 °C gives amino acid (XIII), which is coupled with the quinoline moiety (XI) by a Mitsunobu reaction (PPh_3, DIAD) in THF at 0 °C, affording adduct (XIV), with the proper configuration of the chiral center at position 3 of the cyclopentane.

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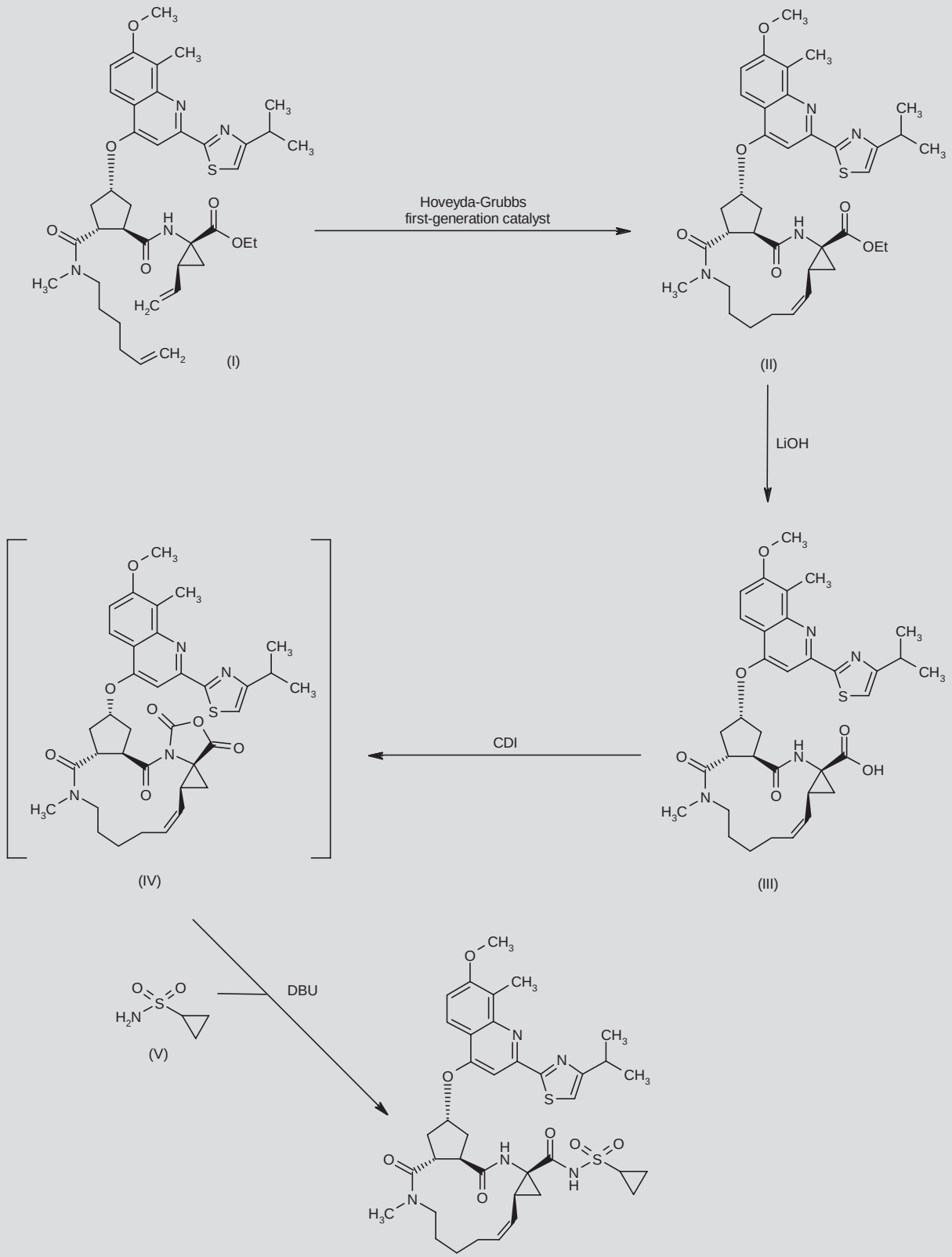
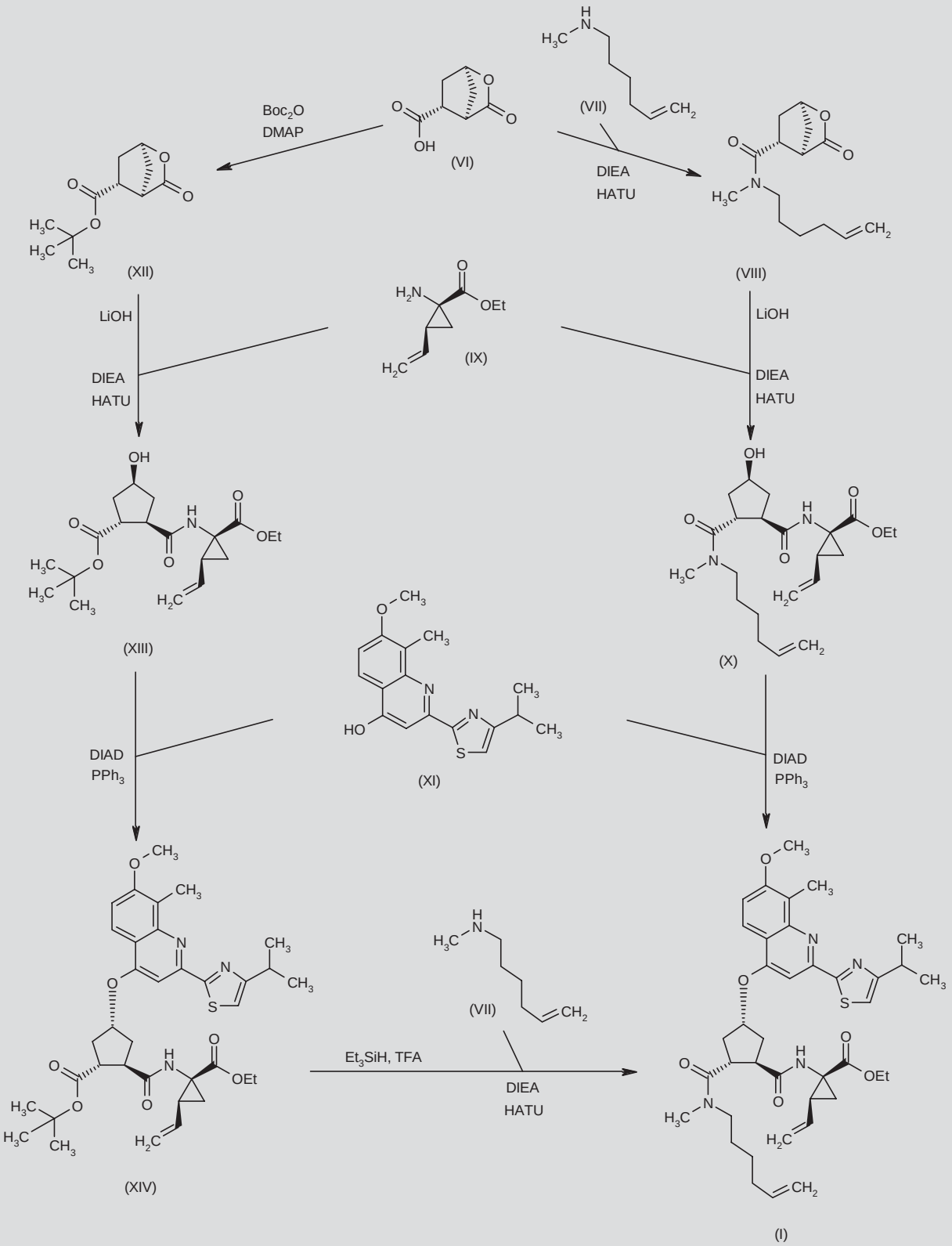
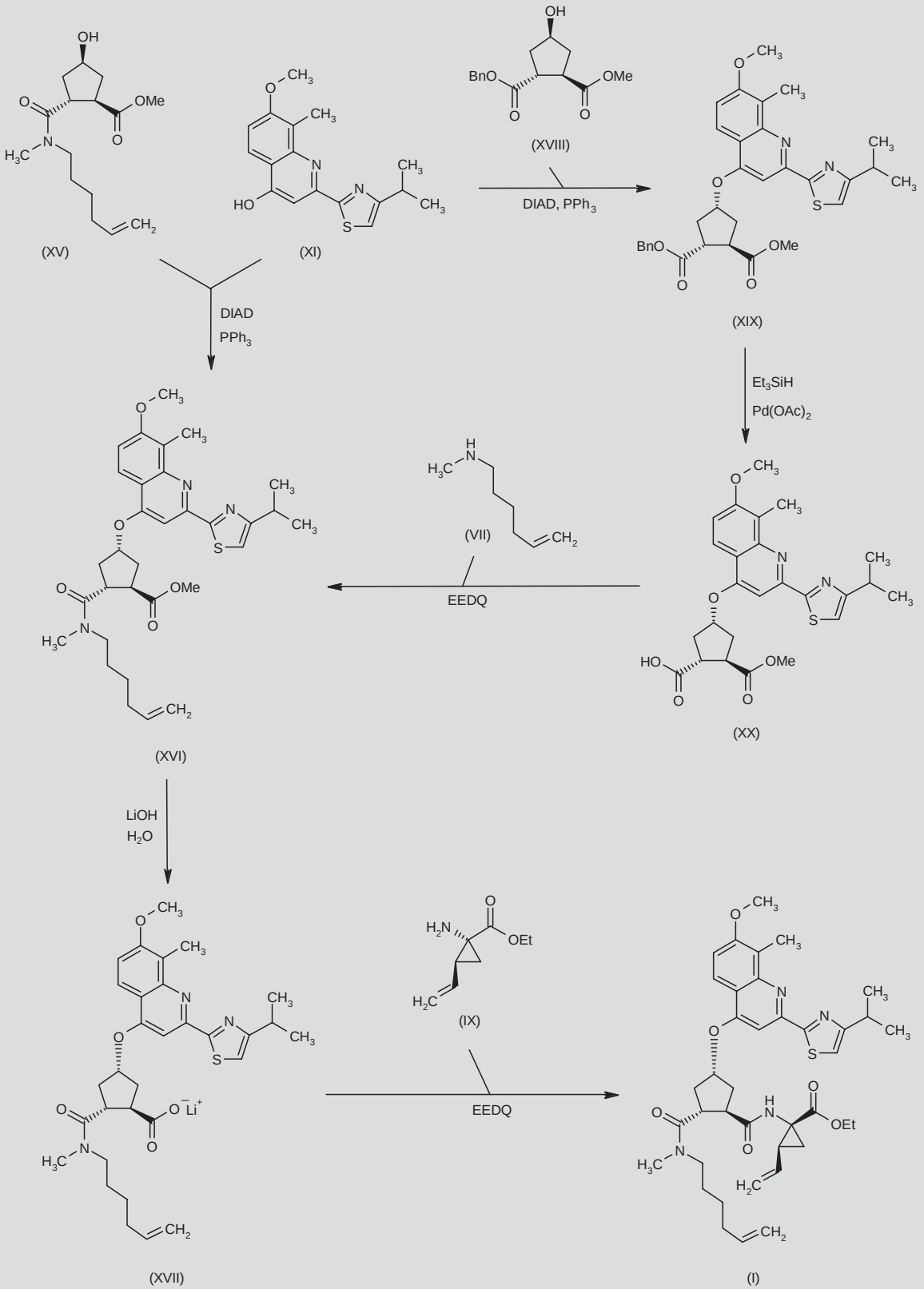
Scheme 1. Synthesis of TMC-435350



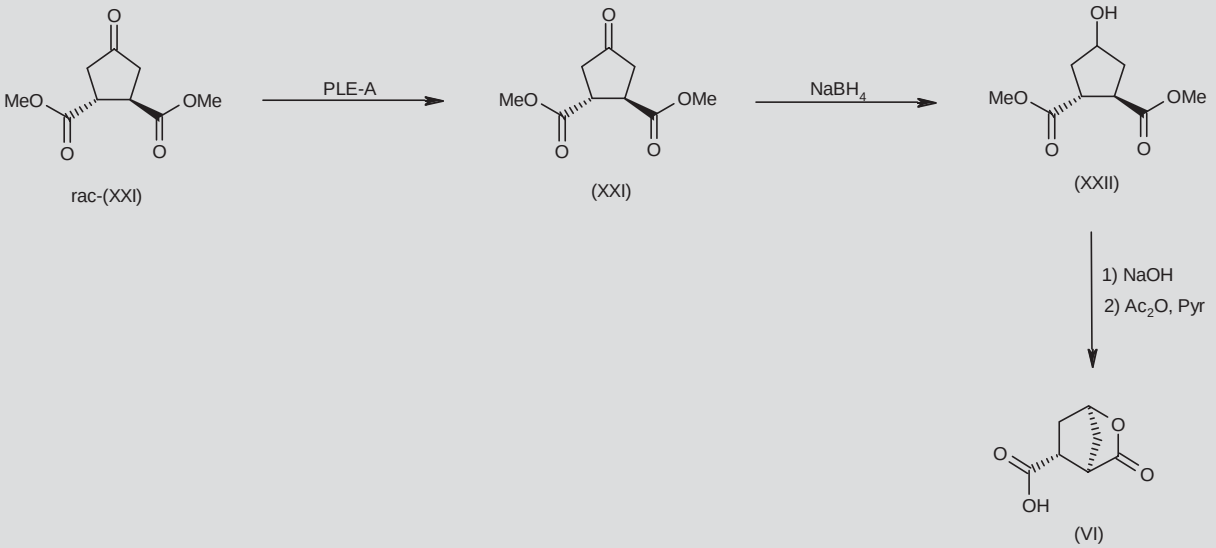
Scheme 2. Synthesis of Intermediate (I)



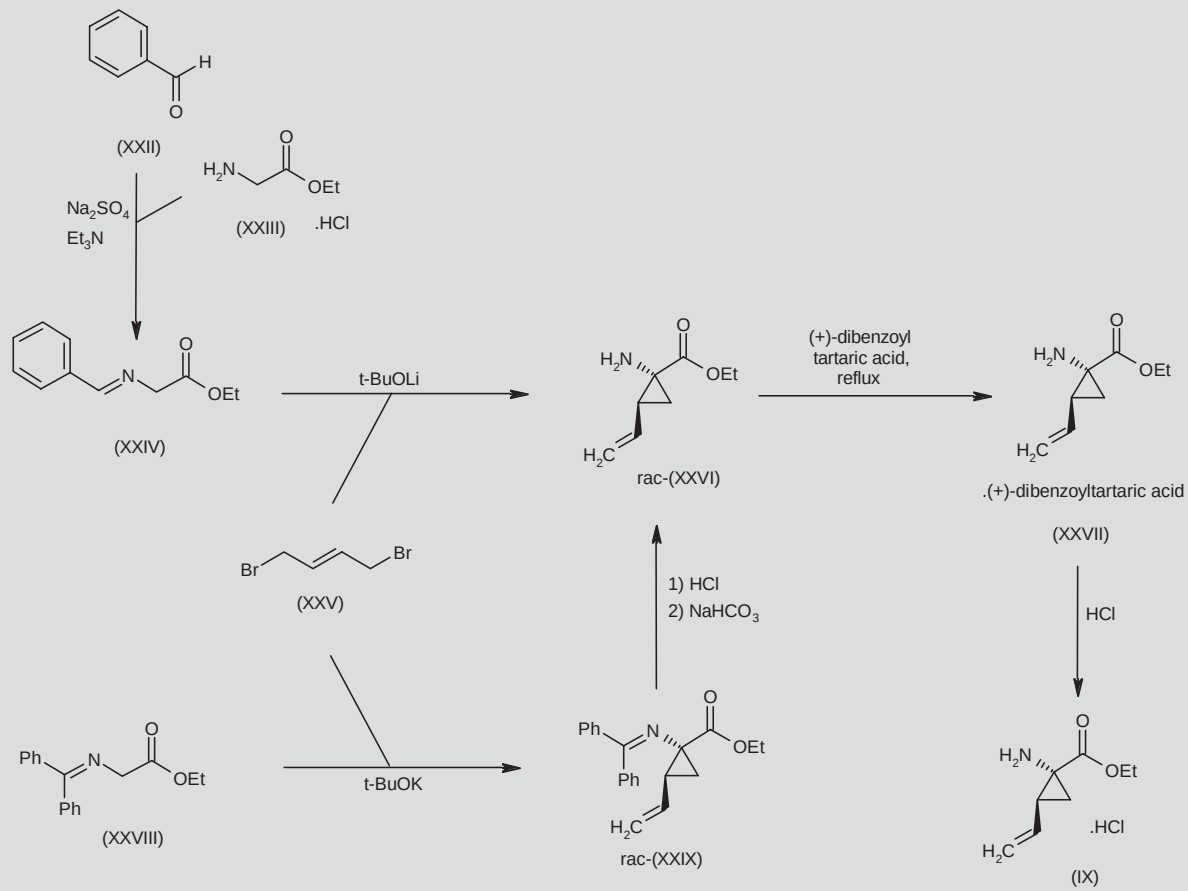
Scheme 3. Synthesis of Intermediate (I)



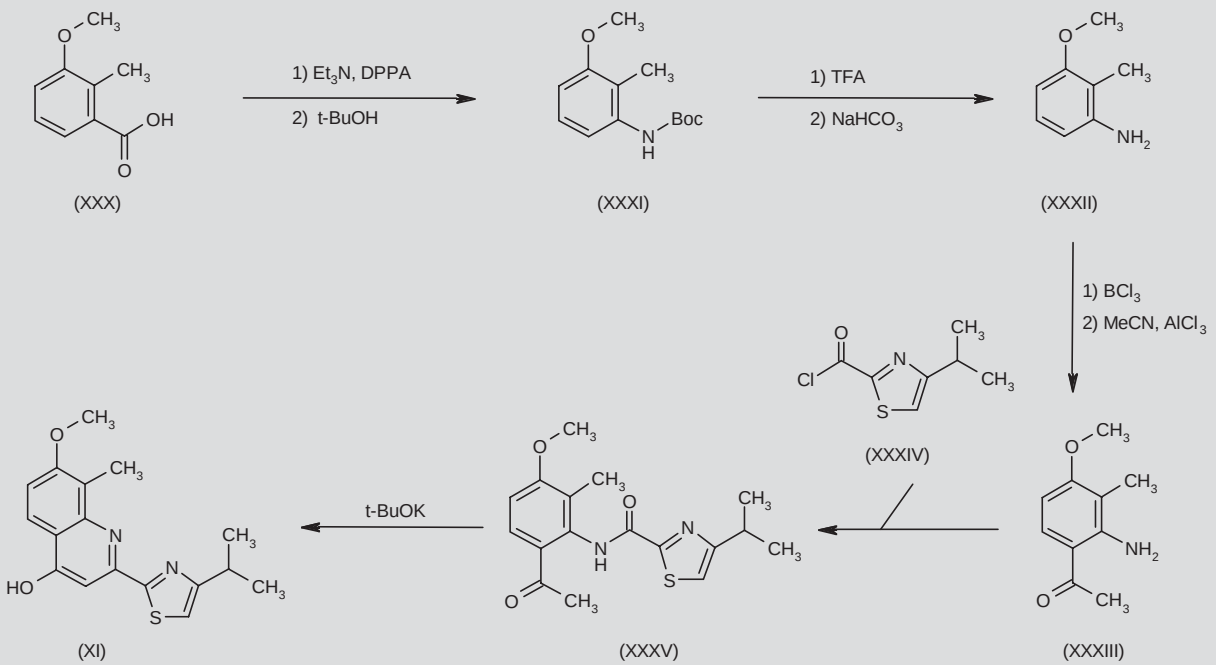
Scheme 4. Synthesis of Intermediate (VI)



Scheme 5. Synthesis of Intermediate (IX)



Scheme 6. Synthesis of Intermediate (XI)



Subsequent deprotection of the *tert*-butyl ester (XIV) with triethylsilane and TFA in CH_2Cl_2 followed by condensation of the resulting acid with alkenylamine (VII) by means of DIAD and HATU in DMF at 0 °C yields the desired noncyclic diene (I) (1). Scheme 2.

Intermediate (I) can also be prepared as follows.

Condensation of the cyclopentanol derivative (XV) with the quinolinol derivative (XI) under Mitsunobu conditions (PPh_3, DIAD) in toluene affords adduct (XVI). Methyl ester (XVI) is hydrolyzed with $LiOH.H_2O$ in THF/H_2O, giving the carboxylate lithium salt (XV), which upon coupling with ethyl 1(*R*)-amino-2(*S*)-vinylcyclopropanecarboxylate (IX) in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in THF/H_2O yields the desired intermediate (I) (4). Scheme 3.

Intermediate (XVI) can also be prepared by condensation of quinolinol derivative (XI) with a different cyclopentanol derivative (XVIII) under Mitsunobu conditions (PPh_3, DIAD) in toluene/THF. The benzyl ester in the resulting adduct (XIX) is converted to the corresponding carboxylic acid (XX) by treatment with triethylsilane and $Pd(OAc)_2$ in refluxing THF or Me-THF. Finally, acid (XX) is amidated with *N*-methylhex-5-enamine (VII) in the presence of EEDQ in Me-THF or THF/Me-THF at 50 °C (4). Scheme 3.

Intermediate (VI) is synthesized as follows.

Enzymatic resolution of *trans*-dimethyl 4-oxocyclopentane-1,2-dicarboxylate (*rac*-[XXI]) with pig liver esterase A (PLE-A) provides the desired (1*R*,2*R*)-enantiomer (XXI) (by selective hydrolysis of the [1*S*,2*S*]-enantiomer) (5). Reduction of ketone (XXI) with $NaBH_4$ affords the *meso*-cyclopentanol derivative (XXII), which is finally treated with NaOH in MeOH and then with acetic anhydride in pyridine to provide the bicyclic lactone (VI) (6). Scheme 4.

Intermediate (IX) is prepared as follows.

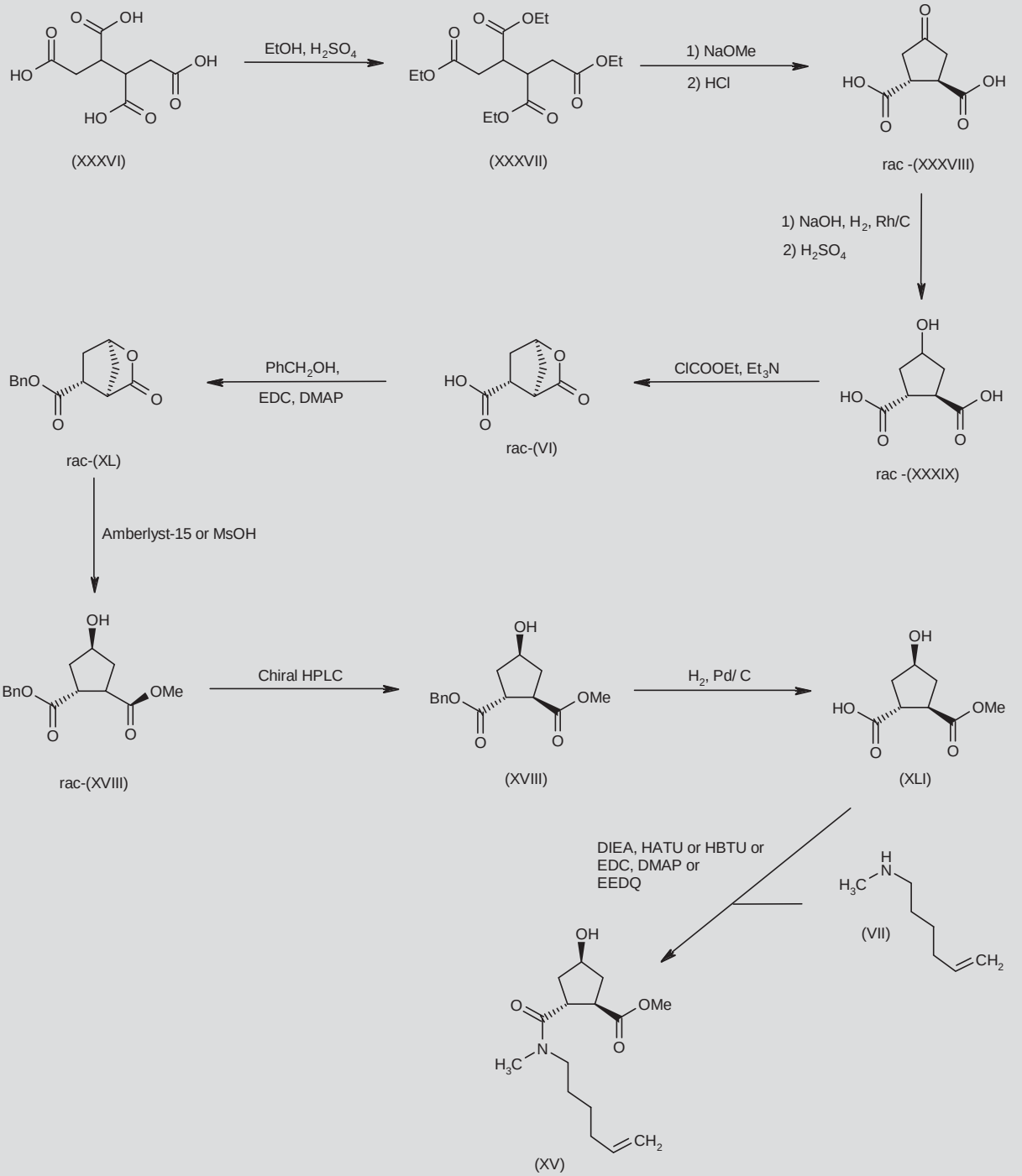
Condensation of benzaldehyde (XXII) with ethyl glycinate hydrochloride (XXIII) in the presence of Na_2SO_4 and Et_3N in *tert*-butyl methyl ether gives ethyl[(benzylidene)amino]acetate (XXIV), which upon reaction with 1,4-dibromo-2-butene (XXV) in the presence of *t*-BuOLi in toluene yields racemic ethyl 1-amino-2-vinylcyclopropanecarboxylate (*rac*-[XXVI]). Resolution of *rac*-(XXVI) by treatment with (+)-dibenzoyltartaric acid in EtOAc gives ethyl 1(*R*)-amino-2(*S*)-vinylcyclopropanecarboxylate (+)-dibenzoyltartrate (XXVII) salt, which is finally treated with HCl in Et_2O (7). Scheme 5.

Alternatively, amino ester (*rac*-[XXVI]) can be prepared by condensation of ethyl ester (XXVIII) with 1,4-dibromo-2-butene (XXV) in the presence of *t*-BuOK in THF, followed by treatment of the resulting imine (*rac*-[XXIX]) with HCl in Et_2O and then with $NaHCO_3$ (7). Scheme 5.

Intermediate (XI) is synthesized as follows.

Curtius rearrangement of 3-methoxy-2-methylbenzoic acid (XXX) by treatment with diphenylphosphonic azide (DPPA) in the presence of Et_3N in toluene at 100 °C followed by *t*-BuOH in refluxing toluene gives *N*-Boc-3-methoxy-2-methylaniline (XXXI), which upon deprotection by means of TFA in CH_2Cl_2 and subsequent basification of the obtained TFA salt with $NaHCO_3$ yields 3-methoxy-2-methylaniline (XXXII). Friedel–Crafts acylation of (XXXII) by a previous treatment with boron trichloride (BCl_3) in

Scheme 7. Synthesis of Intermediate (XV) and (XVIII)



CH_2Cl_2/xylene followed by the addition of acetonitrile and $AlCl_3$ in CH_2Cl_2 at 70 °C furnishes the *ortho*-aminoacetophenone (XXXIII), which upon *N*-acylation with 4-isopropylthiazole-2-carbonyl chloride (XXXIV) in dioxane affords intermediate (XXXV). Finally, intermediate (XXXV) cyclizes in the presence of *t*-BuOK in *t*-BuOH at 100 °C (1-3). Scheme 6.

Intermediates (XV) and (XVIII) are synthesized as follows.

Esterification of 1,2,3,4-butanetetracarboxylic acid (XXXVI) with EtOH in the presence of H_2SO_4 in refluxing toluene yields the corresponding tetraethyl ester (XXXVII), which upon treatment with NaOMe in MeOH followed by HCl in refluxing H_2O yields *trans*-4-oxocyclopentane-1,2-dicarboxylic acid (*rac*-[XXXVIII]). Reduction of intermediate (XXXVIII) with H_2 over Rh/C in the presence of NaOH in H_2O followed by treatment with H_2SO_4 in acetone furnishes *meso*-4-hydroxycyclopentane-1,2-dicarboxylic acid (*rac*-[XXXIX]), which upon treatment with ethyl chloroformate in the presence of Et_3N in THF affords lactone acid (*rac*-[VI]). Acid *rac*-(VI) is esterified with benzyl alcohol in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and DMAP in EtOAc. Benzyl ester (*rac*-[XL]) is treated with Amberlyst-15 or MsOH in MeOH, affording racemic cyclopentanol (*rac*-[XVIII]). Finally, racemate *rac*-(XVIII) is resolved by chiral HPLC, yielding the desired (*R,R,R*)-isomer intermediate (XVIII) (4). Scheme 7.

Intermediate (XV) is prepared from intermediate (XVIII) by hydrogenolysis with H_2 over Pd/C in THF or DMF and subsequent amidation of the resulting carboxylic acid (XLI) with *N*-methylhex-5-enamine (VII) in the presence of DIEA, HATU or *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) in DMF or acetonitrile, or EDC in the presence or absence of DMAP in THF or CH_2Cl_2, or EEDQ in refluxing THF (4). Scheme 7.

BACKGROUND

Hepatitis C is a viral infection of the liver and a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. The World Health Organization (WHO) estimates that, globally, 170 million people (3% of the world's population) are chronically infected with the hepatitis C virus (HCV) and 3-4 million people are newly infected each year (8). Although HCV is less prevalent since the 1990s in the Western world following the introduction of improved blood donor screening programs, needle exchange facilities and education among intravenous drug users, it is still endemic in some regions, including African countries, Egypt, Taiwan, China and Japan (9, 10).

No vaccine is currently available to prevent HCV; therefore, molecular targets involved in the process of viral replication are highly sought after. One important emerging target for therapeutic intervention is the NS3 protease of HCV, which is responsible for the cleavage at the NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B sites of the nonstructural protein. It is essential for viral replication and the formation of infectious viral particles (11).

Tibotec and Medivir are currently developing TMC-435350 (TMC-435) as a potent inhibitor of NS3/4A protease. This agent is involved in an ongoing clinical trial known as OPERA-1, a double-blind, randomized, placebo-controlled phase IIa trial to assess the efficacy, safety, tolerability and pharmacokinetics of four different dose regimens of TMC-435350 (25, 75, 200 and 400 mg daily), given alone or in combination with standard-of-care peginterferon alfa-2a (PegIFN-alfa-2a) and ribavirin (RBV) (12). A nonrandomized, open-label, parallel phase II study of safety and efficacy in Belgium, Germany and Thailand is recruiting patients to assess the activity of TMC-435350 on the replication of HCV genotypes 2, 3, 4, 5 and 6 when administered for 7 days to treatment-naïve patients (13), and a phase I study in Germany determining whether TMC-435350 influences the activity of cytochrome P450 proteins was recently completed (14).

PRECLINICAL PHARMACOLOGY

In vitro studies have demonstrated that TMC-435350 is a potent inhibitor of the HCV NS3/4A protease (K_i = 0.36 nM) and viral replication in Huh-7-Rep cells (EC_{50} = 7.8 nM). This cyclopentane-containing macrocyclic inhibitor also displays low in vitro clearance in human liver microsomes (< 6 µL/min/mg) and high permeability in Caco-2 cells (P_{app} = 8.4 cm/s) (1).

Additional biochemical assays have also reported K_i values of 0.5 and 0.4 nM, respectively, for genotype 1a and 1b NS3/4A proteases. TMC-435350 was also reported to inhibit HCV replication in a cellular assay (subgenomic 1b replicon) with an EC_{50} of 8 nM and a selectivity index of 5,875. The compound was synergistic with interferon alfa and an NS5B inhibitor in the replicon model and additive with ribavirin (15, 16). TMC-435350 has also been shown to bind to NS3 in line with one-step binding kinetics, with a binding affinity of 30.9 nM. IC_{50} values for genotype 2, 4, 5 and 6 proteases have been reported to be < 10-fold higher than the values for genotype 1 (17).

A study has investigated whether TMC-435350 is capable of restoring signaling pathways disrupted by HCV. In vitro data demonstrated antiviral activity in genotype 1b (EC_{50} = 2.8 and 0.27 nmol/L, respectively, for Con-1 and HCV-N) and 1a (EC_{50} = 4.6 and 6.2 nmol/L, respectively, for H77 and H77S.2) replicon cell lines. Furthermore, there was evidence that TMC-435350 can rescue the interferon regulatory factor 3 (IRF-3) signaling pathway via activation of its product, a type 1 interferon (IFN-beta) promoter, but only at concentrations approximately 100-fold greater than the antiviral EC_{50} for genotype 1 HCV (18).

PHARMACOKINETICS AND METABOLISM

Studies in rats have shown that TMC-435350 is extensively distributed to the liver and intestinal tract (tissue/plasma area under the concentration–time curve [AUC] ratio > 35), with an absolute bioavailability of 44% after a single oral administration. Concentrations detected in both plasma and liver at 8 h after dosing were also shown to be above the EC_{99} value measured in the replicon (15, 16).

Pharmacokinetic studies in male Sprague–Dawley rats revealed the following parameters for TMC-435350 following i.v. administration (2 mg/kg): Cl, 0.5 L/h/kg; Vd_{ss}, 0.49 L/kg; AUC, 5.21 µM/h; liver:plasma ratio, 63.5. Upon oral administration (10 mg/kg), the following parameters were reported: AUC, 2.79 µM/h; C_{max}, 0.73 µM; t_{max}, 3 h; $t_{1/2}$, 2.8 h; F, 11%; liver:plasma ratio, 32 (1).

A phase I/II clinical study evaluated the pharmacokinetics of TMC-435350 after single oral doses (50-600 mg) and once-daily oral doses given over 5 days in healthy volunteers (100-400 mg) and HCV genotype 1-infected individuals (HCV+; 200 mg only). Data from this study supported once-daily dosing, with further evidence to suggest that mean exposure in HCV+ subjects is threefold higher than in healthy volunteers and associated with a longer elimination half-life in HCV+ subjects (16 h vs. 41 h, respectively). Pharmacoki-

netic parameters for healthy volunteers compared to HCV+ subjects at 200 mg once daily were reported as follows: C_{max}, 6172 ng/mL vs. 11,470 ng/mL; C_{min}, 1649 ng/mL vs. 6868 ng/mL; and AUC_{24h}, 70,880 ng.h/mL vs. 206,000 ng.h/mL. Further analyses using simulation-based estimates for 25 mg once daily in HCV+ subjects also revealed that trough plasma and liver levels at steady state exceed protein binding-corrected replicon EC_{50} values by > 15- and > 500-fold, respectively, indicating that lower daily doses may also be effective (19, 20).

CLINICAL STUDIES

A placebo-controlled, double-blind phase I study investigated the safety of TMC-435350 100, 200 and 400 mg once daily and 200 mg twice daily (b.i.d.) administered orally with food over a period of 5 days in healthy volunteers (N = 9). No serious or grade 3 or higher adverse events were observed in these subjects. Further open-label assessment of TMC-435350 (200 mg once daily for 5 days) in patients with genotype 1 HCV (n = 4 type 1a; n = 2 type 1b) who had failed prior interferon treatment indicated a rapid decline in HCV RNA in all subjects (median decline at days 3, 5 and 6: 3.5, 3.7 and 3.9 $\log_{10}$ viral IU/mL, respectively). Levels of liver transaminases (ALT/AST) decreased in all subjects during dosing and no viral breakthrough was observed. After halting treatment, plasma levels of HCV RNA had returned to baseline levels in all individuals by week 4 (21).

Interim data for treatment-naïve patients from the ongoing phase IIa OPERA-1 trial (12) were presented. TMC-435350 in combination with PegIFN-alfa-2a/RBV demonstrated potent antiviral activity (viral load reduction of 5.5 and 5.4 $\log_{10}$ IU/mL, respectively, for 75 and 200 mg once daily), with undetectable levels (< 10 IU/mL) in 8 of 9 patients in the 75-mg group and in 7 of 10 patients in the 200-mg group observed after 4 weeks of treatment with triple therapy, and no evidence of viral breakthrough. After 12 weeks of treatment (4 weeks of triple therapy + 8 weeks of PegIFN-alfa-2a/RBV), 6 of 9, 9 of 9 and 10 of 10 patients, respectively, had undetectable virus (< 10 IU/mL) in the 25-, 75- and 200-mg groups. The majority of adverse events were mild to moderate and did not lead to treatment-related discontinuations. No treatment-related hepatic, renal, cardiovascular or hematopoietic adverse events were reported and patients displayed substantial decreases in AST and ALT values (22).

SOURCES

Tibotec Therapeutics (a division of Centocor Ortho Biotech, a subsidiary of Johnson & Johnson) (US); developed in collaboration with Medivir (SE).

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