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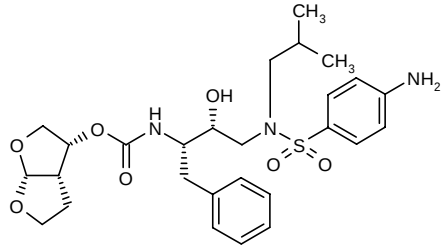
Darunavir

Prop INN; USAN

Anti-HIV Agent
HIV Protease Inhibitor

TMC-114
UIC-94017

N-[3-[*N*-(4-Aminophenylsulfonyl)-*N*-isobutylamino]-1(*S*)-benzyl-2(*R*)-hydroxypropyl]carbamic acid (3*R*,3a*S*,6a*R*)-perhydrofuro[2,3-*b*]furan-3-yl ester



$C_{27}H_{37}N_3O_7S$

Mol wt: 547.6647

CAS: 206361-99-1

EN: 310828

Abstract

Human immunodeficiency virus type 1 (HIV-1) infection remains a major global health problem due to the emergence of drug-resistant strains. Thus, there is an ongoing need for new therapeutics for the long-term management of HIV infection and for acute HIV-1 infection due to drug-resistant strains. HIV-1 protease inhibitors (PIs) have proven to be effective additions to existing antiretroviral regimens. However, despite the success of these agents, the emergence of mutants conferring multidrug resistance (MDR) remains a critical problem. Darunavir is a next-generation nonpeptide PI that exhibits potent antiviral activity with low toxicity *in vitro* and *in vivo*. The agent retains activity against resistant strains and has a low liability for the development of resistance. Darunavir is entering phase III clinical trials and has shown excellent promise as a treatment for HIV-1 infection in treatment-experienced patients.

Synthesis

Darunavir can be prepared by several ways:

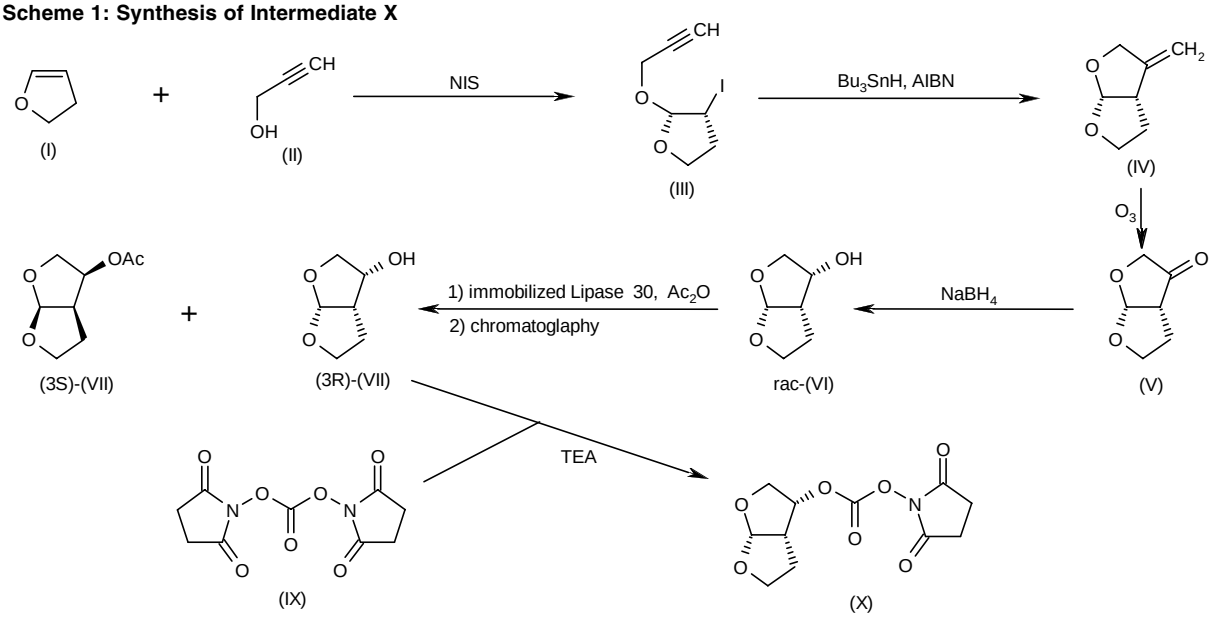
1) Reaction of dihydrofuran (I) with propargyl alcohol (II) and *N*-iodosuccinimide (NIS) gives the propargyl ether (III), which is cyclized by means of tributyltin hydride and AIBN in refluxing toluene to yield the perhydrofuro[2,3-*b*]-furan derivative (IV). Oxidation of the methylene group of compound (IV) with ozone in methanol/dichloromethane affords the bicyclic ketone (V), which is reduced with $NaBH_4$ in ethanol to provide the racemic alcohol (VI). The digestion of racemic (VI) with immobilized Lipase 30 and acetic anhydride in DME provides a mixture of the (3*R*)-alcohol (VII) and the (3*S*)-acetoxy derivative (VIII) that is separated by chromatography. Finally, (3*R*)-alcohol (VII) is condensed with disuccinimidyl carbonate (IX) and TEA in acetonitrile to give the mixed carbonate ester intermediate (X) (1). Scheme 1.

Reaction of butadiene monooxide (XI) with phenylmagnesium bromide (XII) and CuCN in THF gives *trans*-4-phenyl-2-buten-1-ol (XIII), which is enantioselectively epoxidized with $Ti(O\text{-}i\text{-}Pr)_4$, diethyl D-tartrate and *t*-BuOOH to yield the (2*R*,3*R*)-epoxide (XIV). The reaction of compound (XIV) with $Ti(O\text{-}i\text{-}Pr)_4$ and N_3SiMe_3 in refluxing benzene affords the chiral azidodiol (XV), which is epoxidized by means of 2-acetoxyisobutyryl chloride (AcBCl) and NaOMe in THF to provide the chiral azido-epoxide (XVI) (1). Opening of the epoxide ring of compound (XVI) with isobutylamine (XVII) in hot isopropanol gives the secondary amine (XVIII), which is acylated with 4-aminophenylsulfonyl chloride (XIX) and pyridine in dichloromethane to yield the corresponding sulfonamide (XX). Reduction of the azido group of compound (XX) with H_2 over Pd/C in THF/methanol/AcOH affords the expected primary amine (XXI), which is finally condensed with the already reported intermediate (X) and TEA in dichloromethane (1, 2). Scheme 2.

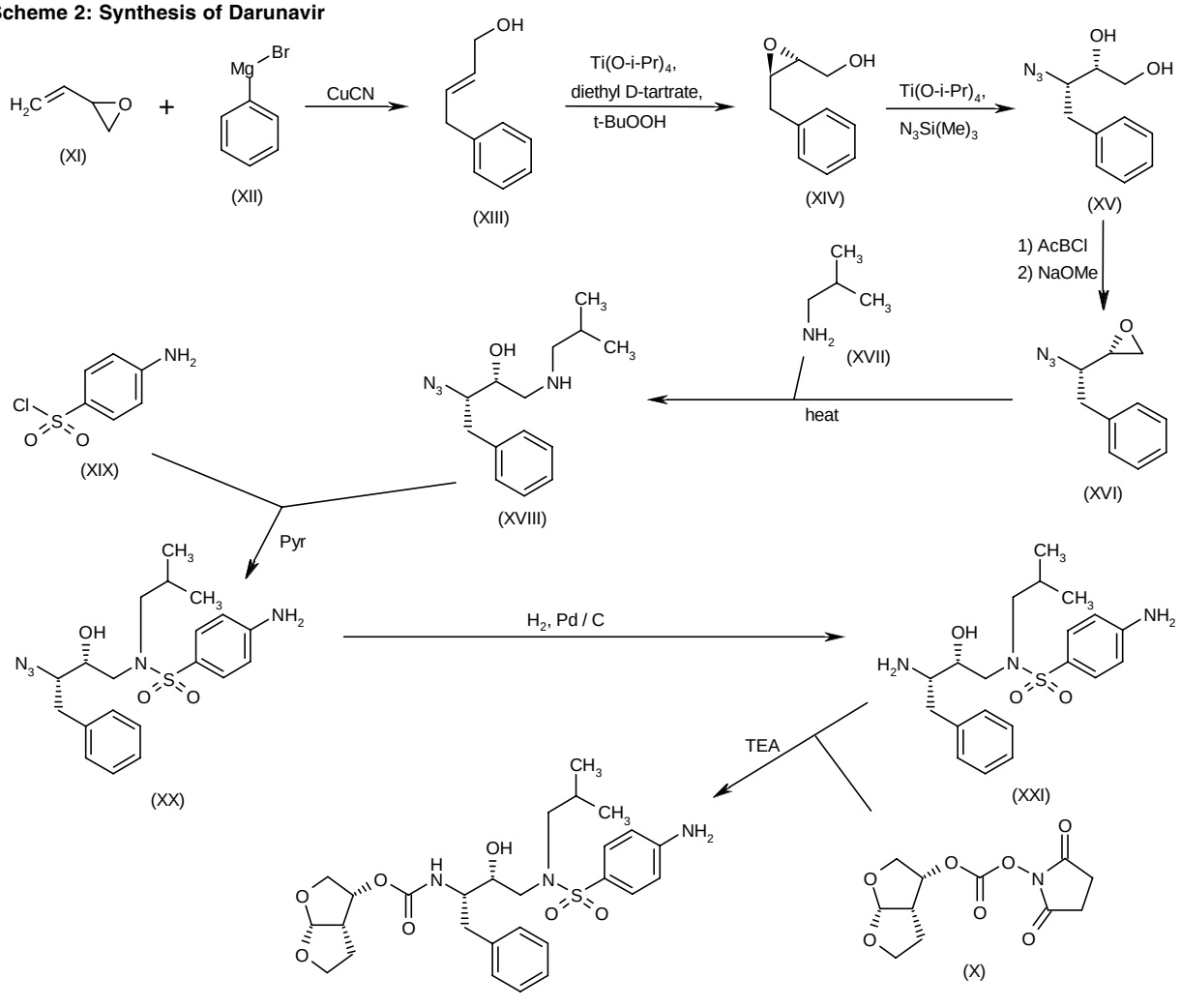
2) Reaction of 2-benzyloxyacetaldehyde (XXII) with vinylmagnesium bromide (XXIII) in THF gives the allyl alcohol (XXIV), which is submitted to optical biocatalytic resolution by means of Lipase PS-30 and isopropenyl acetate (XXV) to obtain a mixture of the (*R*)-acetate (XXVI) and the desired (*S*)-alcohol (XXVII), easily separated by chromatography. The discarded (*R*)-acetate (XXVI) can be recovered by saponification with K_2CO_3 in

L.A. Sorbera, J. Castañer, M. Bayés. Prous Science, P.O. Box 540, 08080, Barcelona, Spain.

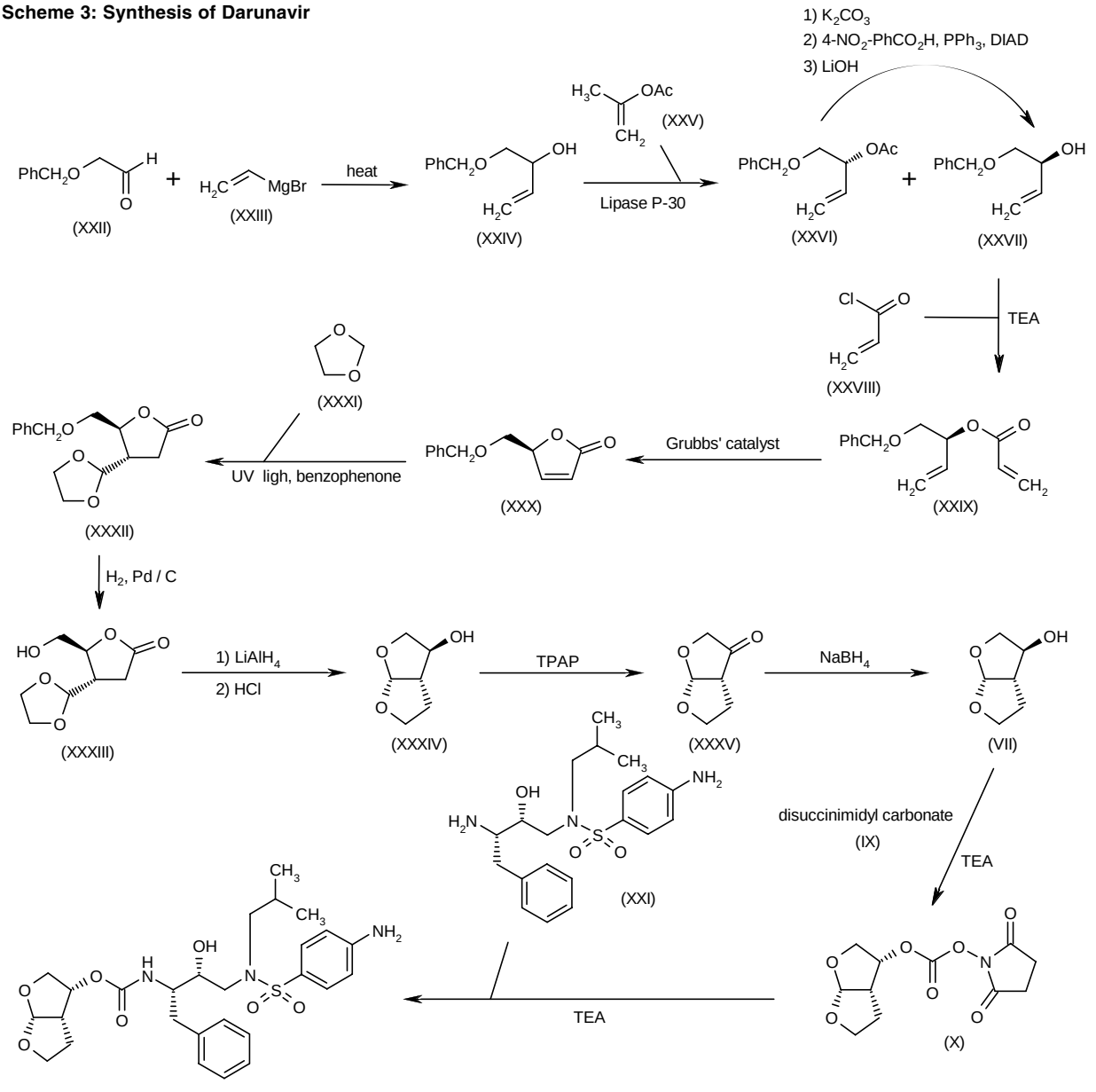
Scheme 1: Synthesis of Intermediate X



Scheme 2: Synthesis of Darunavir



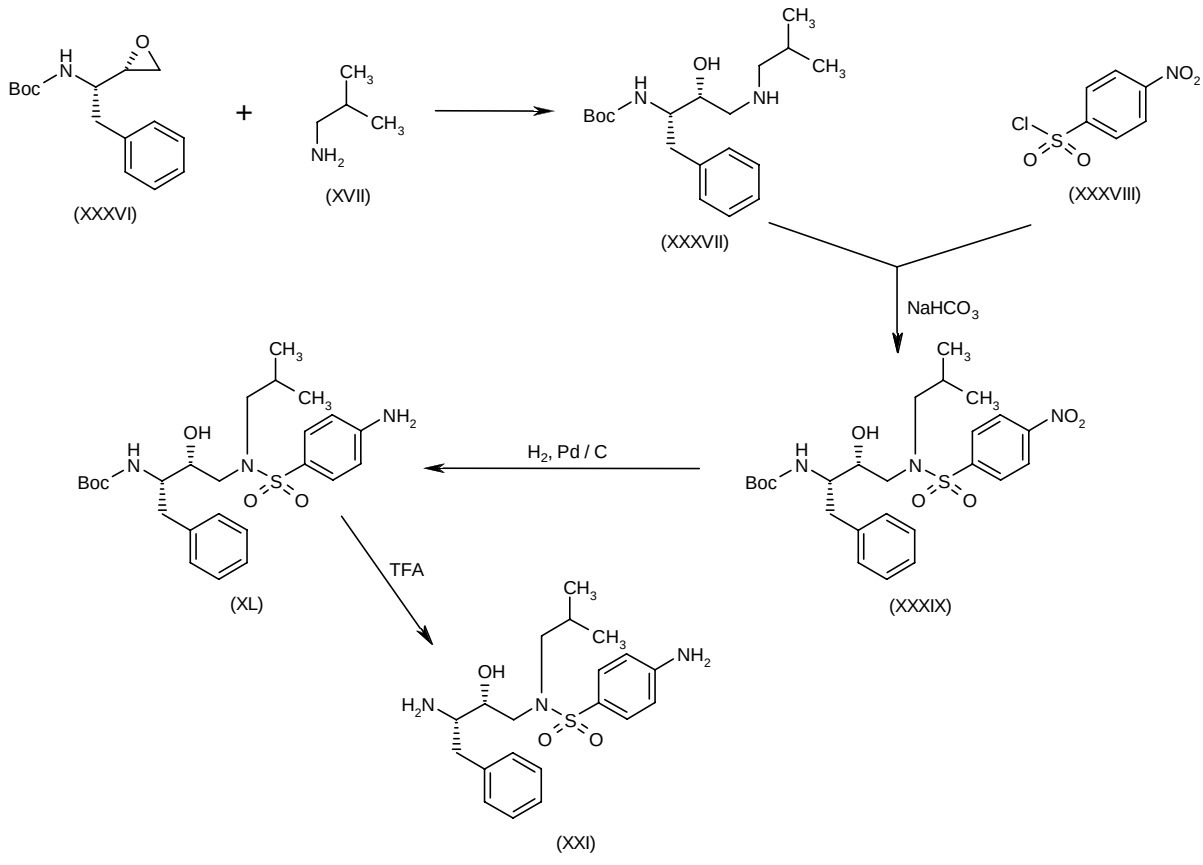
Scheme 3: Synthesis of Darunavir



ethanol, followed by a Mitsunobu inversion reaction with 4-nitrobenzoic acid, PPh_3 and DIAD, and then by hydrolysis with LiOH to afford the desired (*S*)-alcohol (XXVII). Reaction of alcohol (XXVII) with acryloyl chloride (XXVIII) and TEA in dichloromethane gives the corresponding acrylate (XXIX), which is submitted to an olefin metathesis by means of a ruthenium Grubbs' catalyst in hot dichloromethane to yield the chiral dihydrofuranone (XXX). The photochemical addition of 1,3-dioxolane (XXXI) to furanone (XXX) by means of benzophenone and UV light affords the adduct (XXXII), which is debenzylated by catalytic hydrogenation with H_2 over Pd/C in methanol to provide alcohol (XXXIII). Reduction of compound (XXXIII) with $LiAlH_4$ in THF followed by acid-catalyzed cyclization provides the chiral bicyclic alcohol (XXXIV). Epimeric alcohol (XXXIV) is converted to the desired alcohol (VII) by an oxidation/reduction sequence with TPAP oxidation to the ketone (XXXV), followed by reduction with $NaBH_4$ in EtOH or alternatively, by a saponification/Mitsunobu inversion sequence as described before (3, 4). Reaction of bicyclic alcohol (VII) with disuccinimidyl carbonate and TEA in dichloromethane gives the mixed carbonate (X), which is finally condensed with the already obtained 4-aminophenylsulfonamide intermediate (XXI) by means of TEA in dichloromethane (3). Scheme 3.

3) Reaction of the commercially available chiral oxirane (XXXVI) with isobutylamine (XVII) in hot isopropanol

Scheme 4: Synthesis of Intermediate XXI



gives the secondary amine (XXXVII), which is acylated with 4-nitrophenylsulfonyl chloride (XXXVIII) and $NaHCO_3$ in dichloromethane to yield the sulfonamide (XXXIX). Reduction of the nitro group of compound (XXXIX) by means of H_2 over Pd/C affords the 4-aminophenylsulfonamide (XL), which is finally treated with TFA to remove the Boc-protecting group, yielding the sulfonamide intermediate (XXI) (3). Scheme 4.

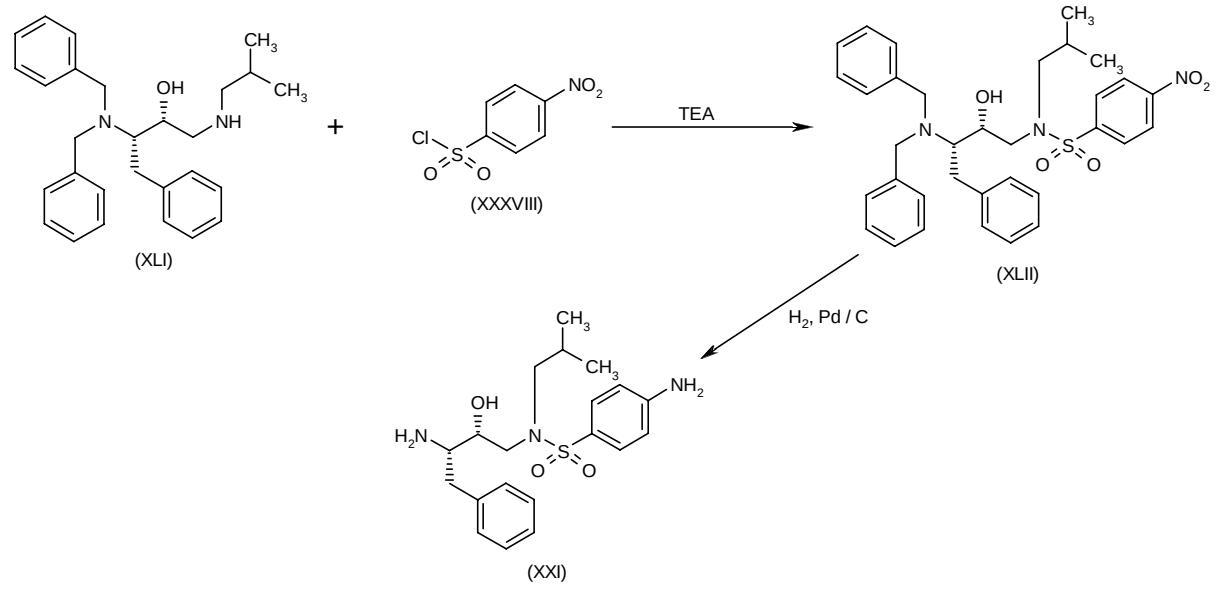
4) Condensation of known 3(*R*)-dibenzylamino-1-isobutylamino-4-phenyl-2(*R*)-butanol (XLI) (5) with 4-nitrobenzenesulfonyl chloride (XXXVIII) by means of TEA in dichloromethane provides the protected sulfonamide (XLII), which is finally converted to the 4-aminophenylsulfonamide intermediate (XXI) by simultaneous deprotection and reduction with H_2 over Pd/C in methanol (6). Scheme 5.

Introduction

According to UNAIDS, there are 39.4 million people worldwide living with human immunodeficiency virus type 1 (HIV-1) infection and acquired immune deficiency syndrome (AIDS). As of December 2004, there were an estimated 37.2 million adults and 2.2 million children under the age of 15 with HIV/AIDS. In addition, in 2004, 4.9 million new HIV infections were diagnosed and 3.1 million people died of HIV/AIDS-related causes. The introduction of highly active antiretroviral therapies (HAARTs) using combinations of agents has resulted in considerable reductions in death rates from HIV-1 infection and AIDS. However, HIV-1 infection remains a major global health problem with an estimated 16,000 new cases diagnosed each day. This is due to the emergence of drug-resistant strains in the presence of suboptimal treatment regimens that do not fully inhibit virus replication. Thus, there is an ongoing requirement for new therapeutics for the long-term management of HIV infection and for acute HIV-1 infection caused by drug-resistant strains (7-11).

HIV-1 protease is required for HIV-1 replication and was identified as an important target for the treatment of HIV-1 infection. HIV-1 protease inhibitors (PIs) were first introduced in late 1995 and represented an excellent addition to existing antiretroviral regimens which include treatment with nucleoside reverse transcriptase inhibitors (NRTIs). The introduction of these agents markedly reduced the mortality rate associated with HIV-1 infection and they are now considered crucial components of HAART (12, 13). However, despite the success of PIs, there is an alarming emergence of mutants which confer

Scheme 5: Synthesis of Intermediate XXI



multidrug resistance (MDR). Thus, there is an immediate need for new agents with broad-spectrum activity against PI-resistant mutants. Several HIV-1 PIs currently under active development are shown in Table I.

Darunavir (TMC-114, UIC-94017) is a next-generation nonpeptide PI that is chemically related to the approved PI amprenavir. The agent exhibited potent antiviral activity, as well as low toxicity, and was shown to have excellent activity against resistant mutants, in addition to a low liability for the development of resistance. Darunavir was therefore chosen for further development as a treatment for HIV-1 infection (2, 6, 14).

Pharmacological Actions

Darunavir exhibited potent activity against a wide spectrum of laboratory strains and primary clinical isolates of HIV-1 (IC_{50} approximately 0.003 μM; IC_{90} approximately 0.009 μM). The pEC_{50} values for the agent against wild-type HIV-1 strain IIIB and PI-resistant strains were 8.39 and 8.14-8.41, respectively. The agent exhibited limited cytotoxicity, with a CC_{50} (50% cytotoxic concentration) of 74 μM against $CD4^+$ MT-2 cells. At concentrations up to 5 μM it was effective in blocking infectivity and replication of HIV-1_{NL4-3} variants exposed to and selected for resistance to saquinavir, indinavir, nelfinavir or ritonavir (IC_{50} = 0.003-0.029 μM). However, it was not as effective against HIV-1_{NL4-3} variants selected for resistance to amprenavir (IC_{50} = 0.22 μM). Potent activity (IC_{50} = 0.003-0.013 μM) was also observed against multi-PI-resistant clinical HIV-1 variant isolates from 7 patients who failed existing anti-HIV regimens after receiving 9-11 anti-HIV-1 agents over the previous 32-83 months (6, 14).

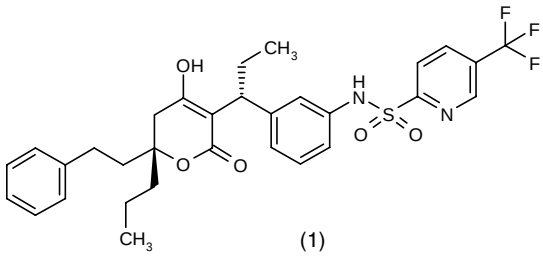
Darunavir was shown to bind more tightly (about 2 orders of magnitude) to wild-type HIV-1 protease than amprenavir (K_d = 0.0045 nM *vs.* 0.39 nM). The excellent binding enthalpy of the agent to wild-type protease appears to be due to the strong interactions between the bis-tetrahydrofuranyl urethane moiety and the main-chain atoms of Asp29 and Asp30. Although darunavir binding to MDR HIV-1 protease was reduced by a factor of 13.3, this binding was 1.5 orders of magnitude tighter than first-generation PIs. Darunavir was also shown to fit predominantly within the substrate envelope, which together with its high affinity may be responsible for the potency seen against MDR strains (15, 16).

Results from *in vitro* selection experiments using MT-4 cell cultures infected with wild-type HIV-1_{LAI} showed that selection of resistant HIV-1 strains was much slower for darunavir as compared to amprenavir, nelfinavir or lopinavir. The selection concentration of darunavir could not be increased over 100 nM for those viruses capable of replicating in the presence of micromolar concentrations of amprenavir, nelfinavir or lopinavir. Strains isolated in the presence of 100 nM darunavir exhibited a low replication capacity and only a 10-fold change in susceptibility to the agent. It was concluded that there is an increased genetic barrier to the development of resistance to darunavir (17).

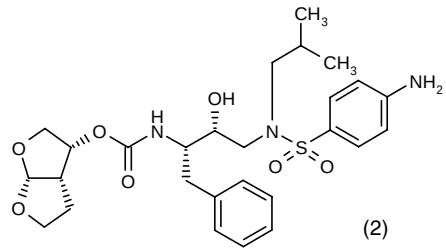
The antiviral activity of darunavir was examined against 4,024 recombinant clinical HIV isolates, of which 1,666 (41%) were resistant to at least 1 approved PI. The median fold change in EC_{50} values for darunavir against these 1,666 isolates was 1.1, which corresponded to an

Table I: HIV protease inhibitors under active development (from Prous Science Integrity®).

Drug	Source	Phase
1. Tipranavir	Behringer Ingelheim (licensed from Pfizer)	Prereg.
2. Darunavir	Tibotec	III
3. 640385/VX-385	GlaxoSmithKline; Vertex	II
4. Kynostatin-272	Japan Energy	II
5. PL-100	Procyon Biopharma	Preclinical
6. PL-337	Procyon Biopharma	Preclinical



(1)



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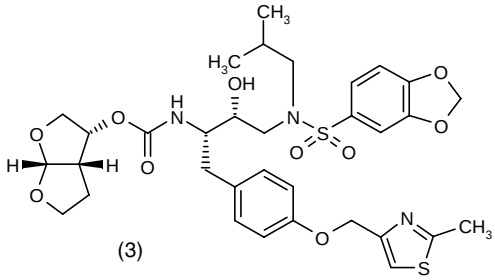

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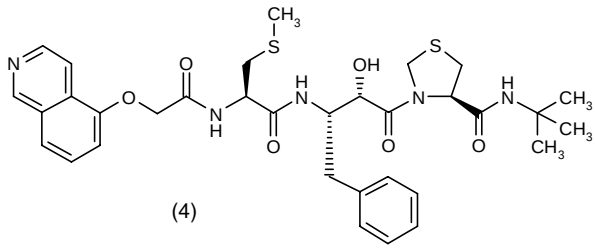

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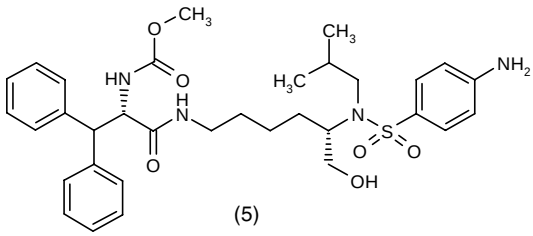

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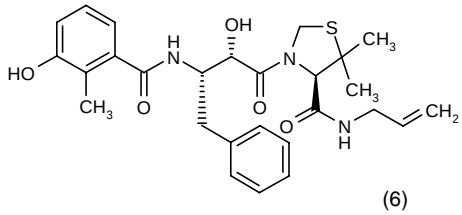

(6)

EC_{50} value of 3.5 nM. Of all the PI-resistant isolates, 80% remained susceptible to darunavir; the median fold change in EC_{50} for the other 20% was 10. Genotypic analysis of 498 of the 1,666 PI-resistant isolates revealed that 1%, 23%, 41%, 31% and 4%, respectively, of the isolates had 0, 1, 2, 3 and 4 primary mutations (D30N, M46I/L, G48V, I50V/L, V82A/F/T/S, I84V, L90M). The median fold change in darunavir EC_{50} for each of these subgroups was < 4. Similar results were obtained in another study in which the antiviral activity of darunavir was examined against more than 4,360 clinical HIV isolates. The median fold change in EC_{50} values for darunavir was < 4 for those isolates with 3 or more primary PI mutations (18, 19).

A further study examined the activity of darunavir against 8 MDR isolates (L10I/F, K20R, L24I, M36I, M46L/I, G48V, I54V, A71I/V, V77I, V82A, I84V, L90M) from patients who received antiretroviral therapy. All isolates were resistant to indinavir, ritonavir, saquinavir, nelfinavir, amprenavir and lopinavir. In contrast, darunavir potently inhibited the replication of all strains with a median EC_{50} value of 5.4 nM (20).

Pharmacokinetics and Metabolism

The metabolic stability of darunavir was examined in liver microsomes from rats, dogs and humans and the results showed comparable stability to other PIs. The percent of parent compound remaining after 30-min incubation at 37 °C was 92%, 80% and 85%, respectively, for rats, dogs and humans. Stability was also observed in oral absorption studies in dogs (80 mg/kg) and rats (20 mg/kg). C_{max}, AUC and $t_{1/2}$ values for the agent were 19,100 ± 5919 and 998.5 ng/ml, 74,081 ± 39,355 and

1578.5 ng·h/ml and 0.7812 ± 0.1994 and 2.06 h, respectively, for dogs and rats (16).

The pharmacokinetics and safety of single oral doses of darunavir (100, 200, 400, 800, 1200, 1600, 2400, 3200 and 4000 mg of a PEG400-based formulation) were assessed in a randomized, double-blind, placebo-controlled, dose-escalating trial conducted in 3 panels of 9 healthy volunteers. All doses were concluded to be safe and well tolerated. The maximum tolerated dose (MTD) was not reached in this study, but further dose increases were not possible due to PEG-related diarrhea. Plasma concentrations initially increased in a more than dose-proportional manner, but no further increases were observed with doses of 2400 and 3200 mg. The mean C_{max} ranged from 14.4 μg/ml to 15.3 μg/ml and the elimination $t_{1/2}$ was about 10 h, irrespective of dose. At doses at or above 800 mg, plasma levels of the agent at 8-12 h postdosing exceeded the protein-adjusted IC_{50} values for PI-resistant isolates. A mild food effect was observed such that the highest bioavailability was seen under fasting conditions (21).

The effect of food on the pharmacokinetics of darunavir (400 mg p.o. as solution and tablets) and ritonavir (100 mg) was studied in 15 healthy volunteers in the fasted and fed states. An increase in darunavir AUC of about 42% was observed in volunteers administered the agent in tablet formulation with food; no changes in systemic exposure were observed with the oral solution between the fed and fasted states. It was concluded that tablet formulations of darunavir should be administered with food (22).

The pharmacokinetics and safety of multiple-dose darunavir (oral PEG formulation for 14 days) with or without low-dose ritonavir were examined in two randomized, dose-escalating phase II trials conducted in a total of 76 healthy volunteers. In the first double-blind, placebo-controlled trial, darunavir was administered at doses of 400 or 800 mg b.i.d. or 800 or 1200 t.i.d. In the second open-label trial, darunavir/ritonavir was given at doses of 200/100, 400/100, 600/200 or 1200/200 mg/day or 300/100 mg b.i.d. The pharmacokinetics of darunavir were improved when coadministered with ritonavir. Darunavir was absorbed rapidly, with C_{max} achieved within 3 h; steady state was reached within 3 days. The C_{max} and C_{min} values obtained in the first trial at day 14 ranged from 2168 ng/ml to 8040 ng/ml and from 14 ng/ml to 142 ng/ml, respectively. The frequency of adverse events appeared to be lower in subjects receiving combination treatment as compared to darunavir alone (23).

The pharmacokinetic interaction between darunavir/ritonavir (300/100 mg b.i.d. for 6 days and a single dose on day 7 followed by a washout period of at least 6 days and subsequent administration on days 1-7 or 8-14) and tenofovir (300 mg/day for 14 days) was evaluated in an open-label, randomized, crossover study in 13 healthy volunteers. Administration of darunavir/ritonavir in the presence or absence of tenofovir was well tolerated. Plasma concentrations of darunavir tended to increase in the presence of tenofovir. However, the increases in darunavir C_{min}, C_{max} and AUC were not significant. About 7% of the total darunavir dose was excreted unchanged in urine in the presence or absence of tenofovir. Similarly, plasma levels of tenofovir were increased in the presence of darunavir/ritonavir, although the increases in C_{min}, C_{max} and AUC for tenofovir were not significant. The increase in the systemic exposure of tenofovir (22%) did not result in an increase in adverse events or laboratory abnormalities. Urinary excretion of unchanged tenofovir was 36% in the presence or absence of darunavir/ritonavir. It was concluded that no dose adjustment is required when these drugs are administered together (24).

A randomized, open-label, crossover study conducted in 16 healthy volunteers examined the effects of darunavir/ritonavir (300/100 mg b.i.d. for 9 days) on the pharmacokinetics of atorvastatin (40 mg/day for 4 days alone or 10 mg/day on days 4-7 in combination). Combination therapy was concluded to be safe and well tolerated, with only mild to moderate adverse events reported. The AUC and C_{max} values for atorvastatin following combination therapy were 15% and 44% lower, respectively, and the C_{min} value was 81% higher as compared to values obtained when the agent was given alone. The main active metabolite after administration of atorvastatin alone was 2-OH-atorvastatin, followed by 4-OH-atorvastatin. These metabolites were not detected after combination treatment. The C_{max} for atorvastatin lactone was 15% lower and the C_{min} was 108% higher with coadministration as compared to atorvastatin monotherapy. Atorvastatin did not alter the pharmacokinetics of darunavir/ritonavir. From these results, an initial dose of 10 mg atorvastatin was recommended, followed, if required, by gradual dose increases depending on clinical responses (25).

Clinical Studies

The antiretroviral activity and safety of darunavir/ritonavir were examined in an open, randomized phase IIa study enrolling 50 multiple PI-experienced patients (median baseline plasma HIV-1 RNA = 4.3 $\log_{10}$ copies/ml; median baseline $CD4^+$ cell count = 297/μl). Patients were administered darunavir/ritonavir (group A: 300/100 mg b.i.d.; group B: 600/100 mg b.i.d.; group C: 900/100 mg/day) for 14 days in place of their failing PIs (other antiretrovirals were unchanged) or continued on their failing regimen (control group D). Treatment with darunavir/ritonavir was generally well tolerated. The most common adverse events were gastrointestinal (GI) disturbances, with 1 patient each in groups C and B discontinuing due to GI discomfort and a serious case of hepatitis, respectively. In groups A, B and C, 2, 2 and 1 patients, respectively, had grade 3/4 increases in ALT, AST and GGT, and 1 patient in group D had grade 3 elevations in AST. Intent-to-treat analysis showed that after 2 weeks, the median change in plasma HIV-1 RNA for groups A, B, C and D was –1.24, –1.50, –1.13 and +0.02 $\log_{10}$ copies/ml, respectively. Of the patients in groups A, B, C and D,

Table II: Clinical studies of darunavir (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
HIV infection	Randomized Open	Darunavir, 300 mg b.i.d. + Ritonavir, 100 mg b.i.d. + Baseline therapy [no protease inhibitors] x 14 d (n=13) Darunavir, 600 mg b.i.d. + Ritonavir, 100 mg b.i.d. + Baseline therapy [no protease inhibitors] x 14 d (n=12) Darunavir, 900 mg b.i.d. + Ritonavir, 100 mg b.i.d. + Baseline therapy [no protease inhibitors] x 14 d (n=13) Baseline antiretroviral therapy x 14 d (n=12)	50	All three darunavir/ritonavir regimens were significantly more effective than baseline regimens in reducing the viral load in patients with HIV infection, and well tolerated	27, 29

69%, 92%, 69% and 17%, respectively, had a reduction in HIV-1 RNA of at least 1.0 $\log_{10}$ copies/ml, and of all patients receiving darunavir/ritonavir (groups A, B and C), 97% had a reduction of at least 0.50 $\log_{10}$ copies/ml. The maximum and median changes in HIV-1 RNA for patients receiving darunavir/ritonavir were –2.49 and –1.35 $\log_{10}$ copies/ml, respectively. The median C_{min} and AUC for darunavir on day 14 for groups A, B and C were 1.2 μg/ml and 53.3 μg·h/ml, 1.4 μg/ml and 60.4 μg·h/ml, and 1.6 μg/ml and 67.9 μg·h/ml, respectively. Further analysis showed that genotypic, phenotypic, pharmacokinetic and interquartile parameters did not predict viral load changes on day 14. These results indicate that the doses used produced sufficient exposure to overcome broad resistance to PIs (26-29) (Table II).

Analysis of data at 24 weeks from two 96-week, multinational, dose-finding phase IIb studies including 497 HIV-1-infected patients who had received at least 3 classes of antiretrovirals and who had 1 or more primary PI mutations and limited treatment options (median baseline HIV-1 RNA = 4.6 $\log_{10}$ copies/ml; $CD4^+$ cell count = 141 cells/μl) demonstrated the antiretroviral efficacy of darunavir/ritonavir (400/100 or 800/100 once daily or 40/100 or 600/100 b.i.d.) as compared to a control group receiving investigator-selected control PIs. Treatment was generally well tolerated. The incidence of adverse events was similar in all treatment groups, with severe adverse event rates of 10%, 17%, 16% and 9%, respectively, and respective rates for grade 3 and 4 adverse events of 23%, 26%, 26% and 26% at week 24. The most common adverse events in all darunavir/ritonavir groups were headache (17% *vs.* 23% in controls) and diarrhea (14% *vs.* 20% in controls). At week 24, the mean reductions in plasma HIV-1 RNA were 1.28, 1.43, 1.47 and 1.85 $\log_{10}$ copies/ml for the respective dose groups. The mean change in $CD4^+$ cell counts in the group receiving a dose of 600/100 mg b.i.d. was +75 cells/μl. At 24 weeks, 30-47% of all patients receiving darunavir/ritonavir had undetectable virus levels (< 50 copies/ml) as compared to only 10% in the control group. At 24 weeks, the recommended dose of darunavir/ritonavir for phase III trials in treatment-experienced patients was 600/100 mg b.i.d. These studies will continue to 96 weeks (30).

Darunavir in combination with ritonavir is entering phase III development for the treatment of HIV-1 infection in treatment-experienced patients (31).

Source

Tibotec (BE, US) (a subsidiary of Johnson & Johnson).

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