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CARFILZOMIB

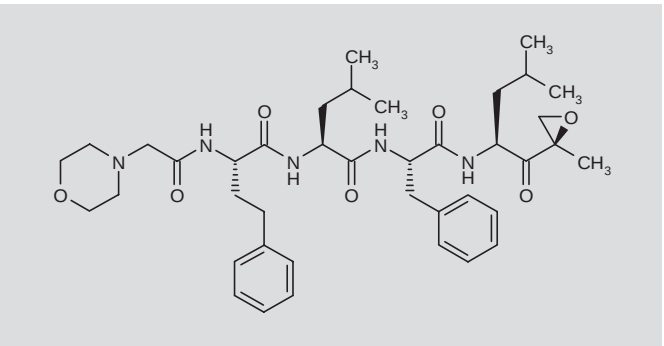
Rec INN; USAN

Proteasome Inhibitor
Oncolytic

PR-171

N-[2(*S*)-[2-(4-Morpholinyl)acetamido]-4-phenylbutyryl]-L-leucyl-*N*^{1}-[3-methyl-1(*S*)-[2(*R*)-methyloxiran-2-ylcarbonyl]butyl]-L-phenylalaninamide

InChI: 1S/C40H57N5O7/c1-27(2)22-32(36(47)40(5)26-52-40)42-39(50)34(24-30-14-10-7-11-15-30)44-38(49)33(23-28(3)4)43-37(48)31(17-16-29-12-8-6-9-13-29)41-35(46)25-45-18-20-51-21-19-45/h6-15,27-28,31-34H,16-26H2,1-5H3,(H,41,46)(H,42,50)(H,43,48)(H,44,49)/t31-,32-,33-,34-,40+/m0/s1



$C_{40}H_{57}N_5O_7$
Mol wt: 719.9099
CAS: 868540-17-4
EN: 413092

ABSTRACT

Proteasome inhibitors are a new class of drugs that have been shown to be cytotoxic and to induce apoptosis in tumor cells, particularly multiple myeloma (MM). In a bid to overcome the dose-limiting toxicity and adverse events observed with nonselective proteasome inhibitors, Proteolix is currently developing a novel, irreversible, epoxomicin-related proteasome inhibitor, carfilzomib (PR-171). Pharmacodynamic studies have suggested that this agent spares constitutive proteasome function, showing more selective inhibition of the immunoproteasomes of hematological tumor cells. Clinical data presented to date indicate that carfilzomib provides sustained clinical benefits in relapsed and refractory MM patients and is active as a single agent and in combination regimens. Carfilzomib is presently undergoing phase Ib/II clinical investigation in the U.S.

SYNTHESIS

Carfilzomib can be prepared as follows.

Condensation of the chloroacetyl tripeptide (I) with morpholine (II) by means of KI in THF gives the tetrapeptide derivative (III), which is hydrolyzed with LiOH in MeOH/H_2O to yield the corresponding carboxylic acid (IV) (1). Alternatively, reaction of the trifluoroacetate salt of tripeptide (V) with 4-morpholinoacetic acid (VI) in the presence of DIEA and PyBOP in DMF affords the tetrapeptide benzyl ester (VII), which is then debenzylated by means of H_2 over Pd/C in MeOH/EtOAc to give the carboxylic acid (IV) (2-4). Finally, the carboxylic acid building block (IV) is coupled with either 2(*S*)-amino-4-methyl-1-[2(*R*)-methyloxiran-2-yl]pentan-1-one trifluoroacetate salt (VIII) in the presence of DIEA, HOBt and PyBOP in acetonitrile (2-4), or BOP in DMF, or HBTU in DMF or acetonitrile (1), or with the formic acid salt (IX) by means of DIEA, HOBt and HBTU in acetonitrile (1) Scheme 1. The corresponding citrate salt is prepared by treatment of carfilzomib with citric acid in THF (1).

The chloroacetyl tripeptide precursor (I) is obtained as follows.

Condensation of *N*-Boc-L-leucine (X) with L-phenylalanine methyl ester (XI) in the presence of DIEA, HOBt and BOP in DMF gives the protected dipeptide (XII). Dipeptide (XII) is then deprotected by means of TFA in CH_2Cl_2 to yield L-Leu-L-Phe-OMe trifluoroacetate salt (XIII), which without isolation is then condensed with *N*-Boc-L-homophenylalanine (XIV) in the presence of DIEA, HOBt and BOP in acetonitrile to yield the protected tripeptide (XV) . Then, *N*-deprotection of peptide (XV) by means of TFA in CH_2Cl_2 gives tripeptide (XVI), which is finally coupled with chloroacetyl chloride (XVII) in the presence of DIEA in DMF (1). Scheme 2.

The tripeptide benzyl ester precursor (IV) is prepared by the following procedure. Condensation of *N*-Boc-L-leucine (X) with L-phenylalanine benzyl ester (XVIII) in the presence of DIEA, HOBt and PyBOP in acetonitrile gives the protected dipeptide (XIX), which upon deprotection by means of TFA in CH_2Cl_2 yields dipeptide (XX). Without isolation, intermediate (XX) is then condensed with *N*-Boc-L-homophenylalanine (XIV) in the presence of DEEA (diethyl-aminoethanol),

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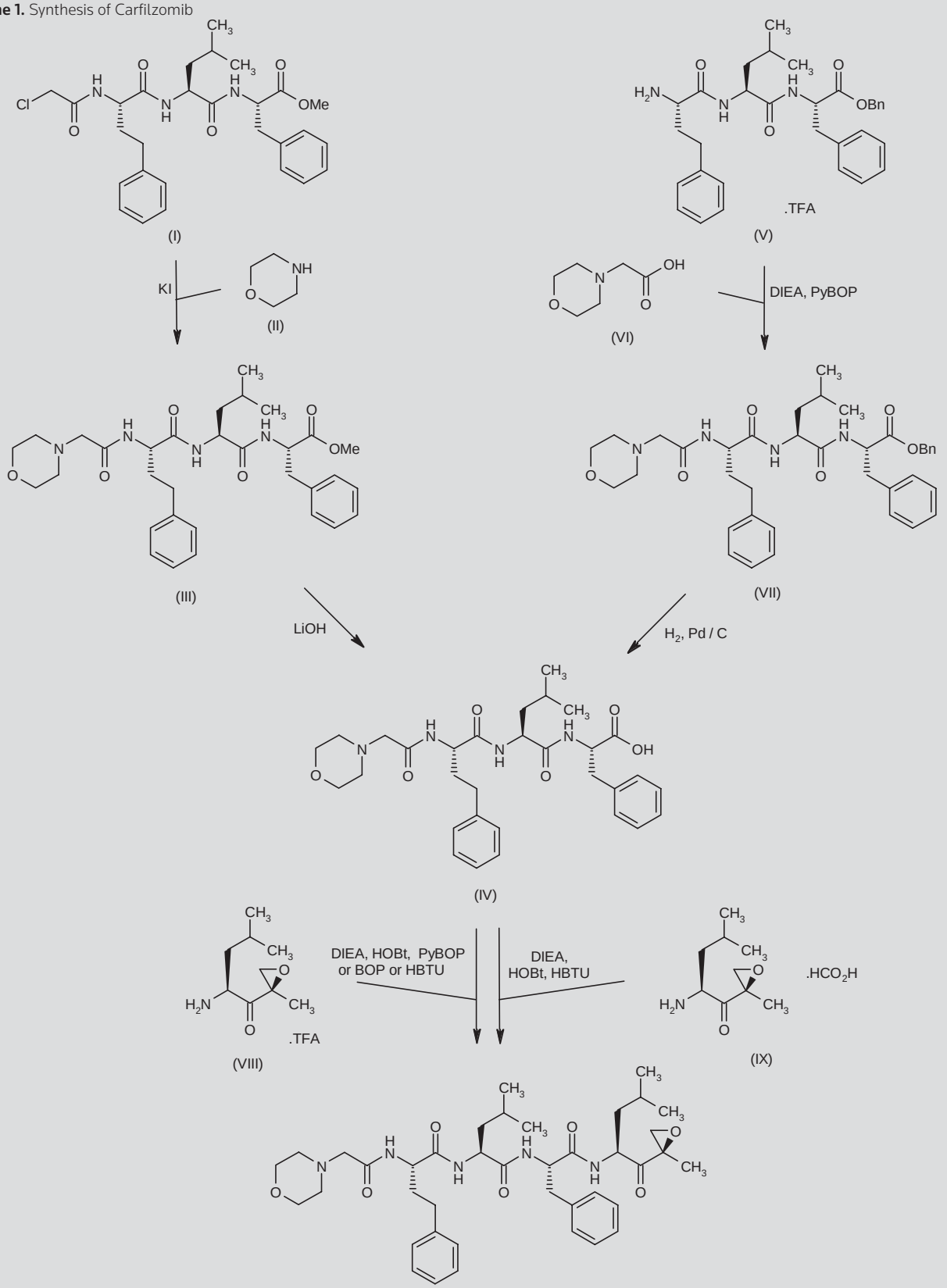
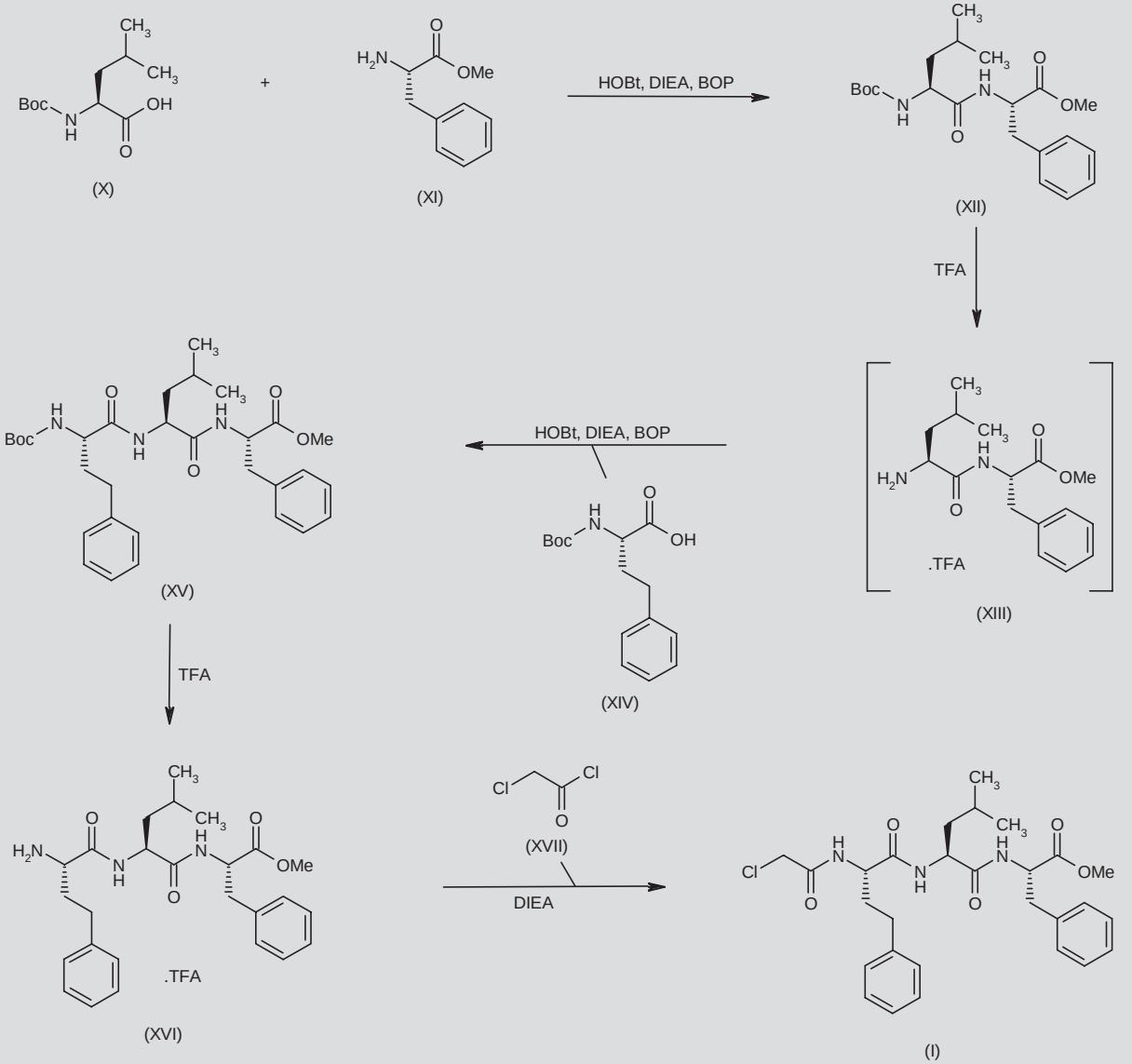
Scheme 1. Synthesis of Carfilzomib



Scheme 2. Synthesis of Intermediate (I)



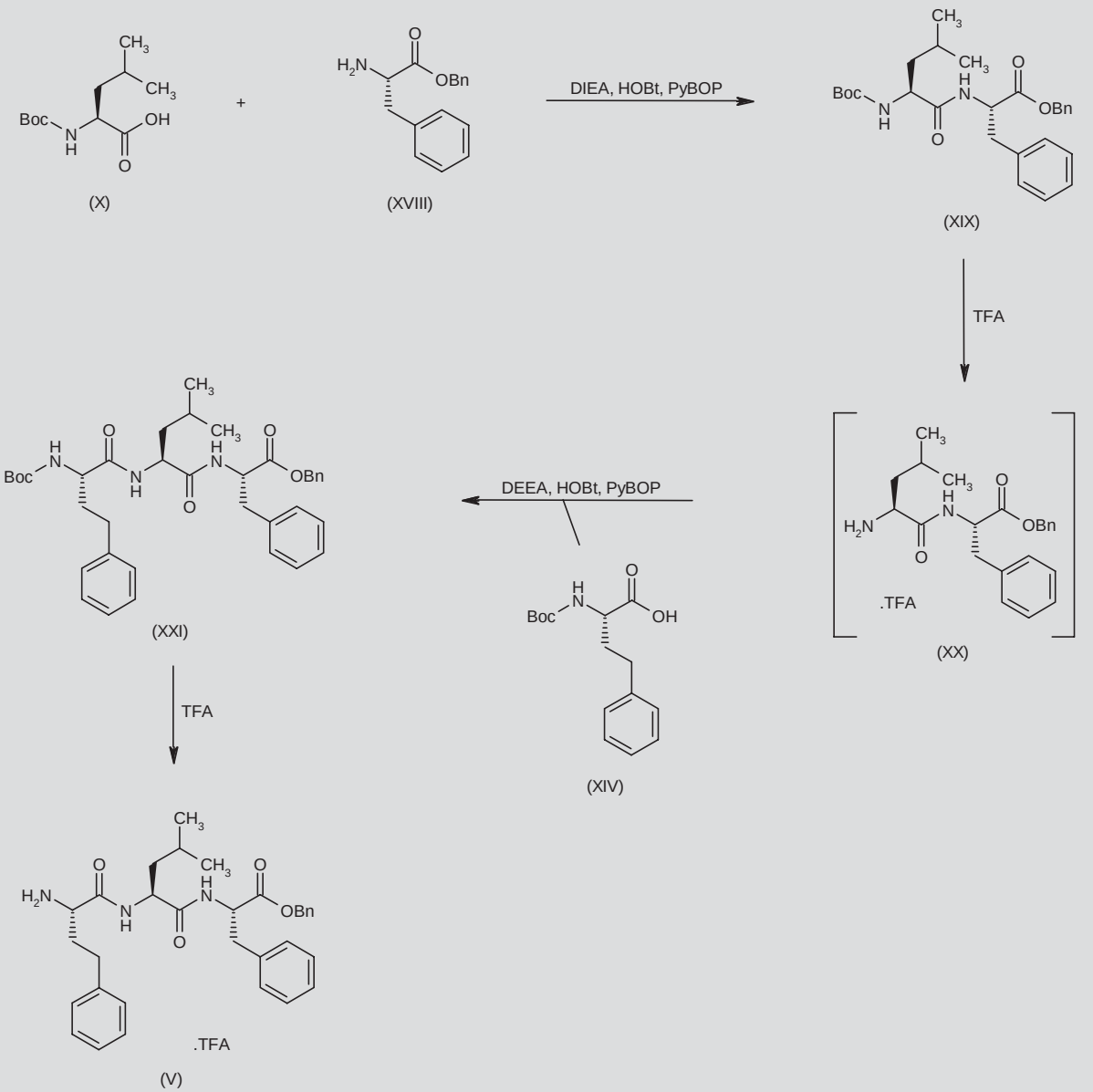
HOBt and PyBOP in acetonitrile to provide protected tripeptide (XXI) (*2*, 4), which is finally *N*-deprotected by means of TFA in CH_2Cl_2 (*2*-4). Scheme 3.

The epoxide building blocks (VIII) and (IX) are prepared starting from *N*-Cbz-4(*S*)-amino-*2*,6-dimethyl-1-hepten-3-one (XXII). Reduction of ketone (XXII) with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ in MeOH gives a diastereomeric mixture of allylic alcohols (XXIIIa) and (XXIIIb), which by epoxidation with mCPBA in CH_2Cl_2 or $VO(acac)_2$ and *t*-BuOOH also in CH_2Cl_2 yields a mixture of oxiranes (XXIVa) and (XXIVb). Oxidation of the mixture of alcohols (XXIVa) and (XXIVb) by means of NMO and TPAP in CH_2Cl_2 or Dess Martin periodinane in DMSO or by Swern oxidation (treatment with DMSO and $[COCl]_2$ in CH_2Cl_2, followed by Et_3N in CH_2Cl_2), and then chromatographic separation of the diastereomers affords *N*-Cbz-*2*(*S*)-amino-4-methyl-1-[*2*(*R*)-methyloxiran-*2*-yl]pentan-1-one (XXV) (5, 6), which is then deprotected by transfer hydrogenation with HCOOH and Pd/C or by catalytic hydrogenation over Pd/C in the presence of TFA, providing the corresponding formic acid salt (IX) and TFA salt (VIII) (1). Alternatively, deprotection of the epoxy amino acid (XXVI) by means of TFA also gives the TFA salt (VIII) (1, 6). Scheme 4.

The epoxy amino acid precursor (XXVI) can be obtained as follows.

Condensation of *N*-Boc-leucine (X) with *N,O*-dimethylhydroxylamine hydrochloride by means of *i*-BuOCOCl, NMM and Et_3N in CH_2Cl_2 gives *N*-Boc-L-leucine Weinreb amide (XXVII) (1), which is then coupled with

Scheme 3. Synthesis of Intermediate (IV)

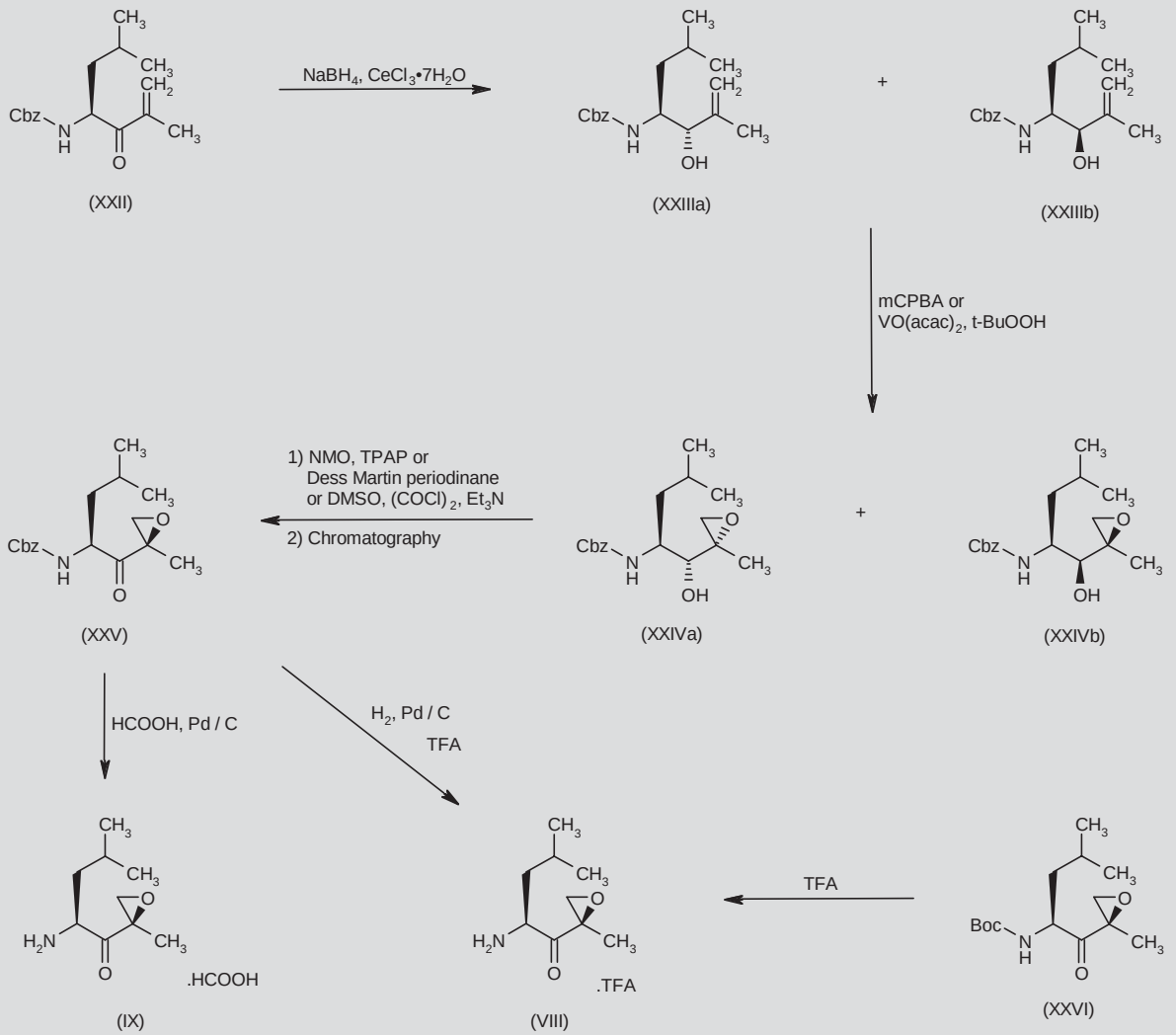


isopropenyl magnesium bromide (XXVIII) in THF (1) or 2-bromopropene (XXIX) in the presence of BuLi in Et_2O at –78 °C (6) to yield the heptenone derivative (XXX) (1, 6). Reduction of heptenone (XXX) with $NaBH_4$ and $CeCl_3{\cdot}7H_2O$ in MeOH results in a diastereomeric mixture of allylic alcohols (XXXIa) and (XXXIb), which is then epoxidized by treatment with mCPBA in CH_2Cl_2 to afford a mixture of oxiranes (XXXIIa) and (XXXIIb). Finally, this mixture is oxidized with Dess Martin periodinane in acetonitrile, followed by chromatographic separation (1). Compound (XXVI) can also be prepared by epoxidation of heptenone derivative (XXX) with H_2O_2 in the presence of PhCN and DIEA in MeOH (6) or NaOCl in pyridine or $Ca(OCl)_2$ in NMP (1), followed by separation by means of column chromatography (Scheme 5).

BACKGROUND

More than 100,000 cases of hematological cancers (leukemia, lymphoma and myeloma) were diagnosed in the U.S. in 2005, with over 53,000 deaths from these cancers reported during that year (7). Proteasome inhibitors represent a new class of antineoplastic drugs for the treatment of such hematological malignancies. A proteasome is a multicatalytic proteinase complex responsible for the degradation of most intracellular proteins, including proteins crucial to cell cycle regulation and apoptosis (8, 9). Clinical validation of the proteasome as a therapeutic target in oncology has been provided by bortezomib (PS-341, Velcade™), a proteasome inhibitor that has proven efficacy in multiple myeloma (MM) and non-Hodgkin's lym-

Scheme 4. Synthesis of Intermediates (VIII) and (IX)

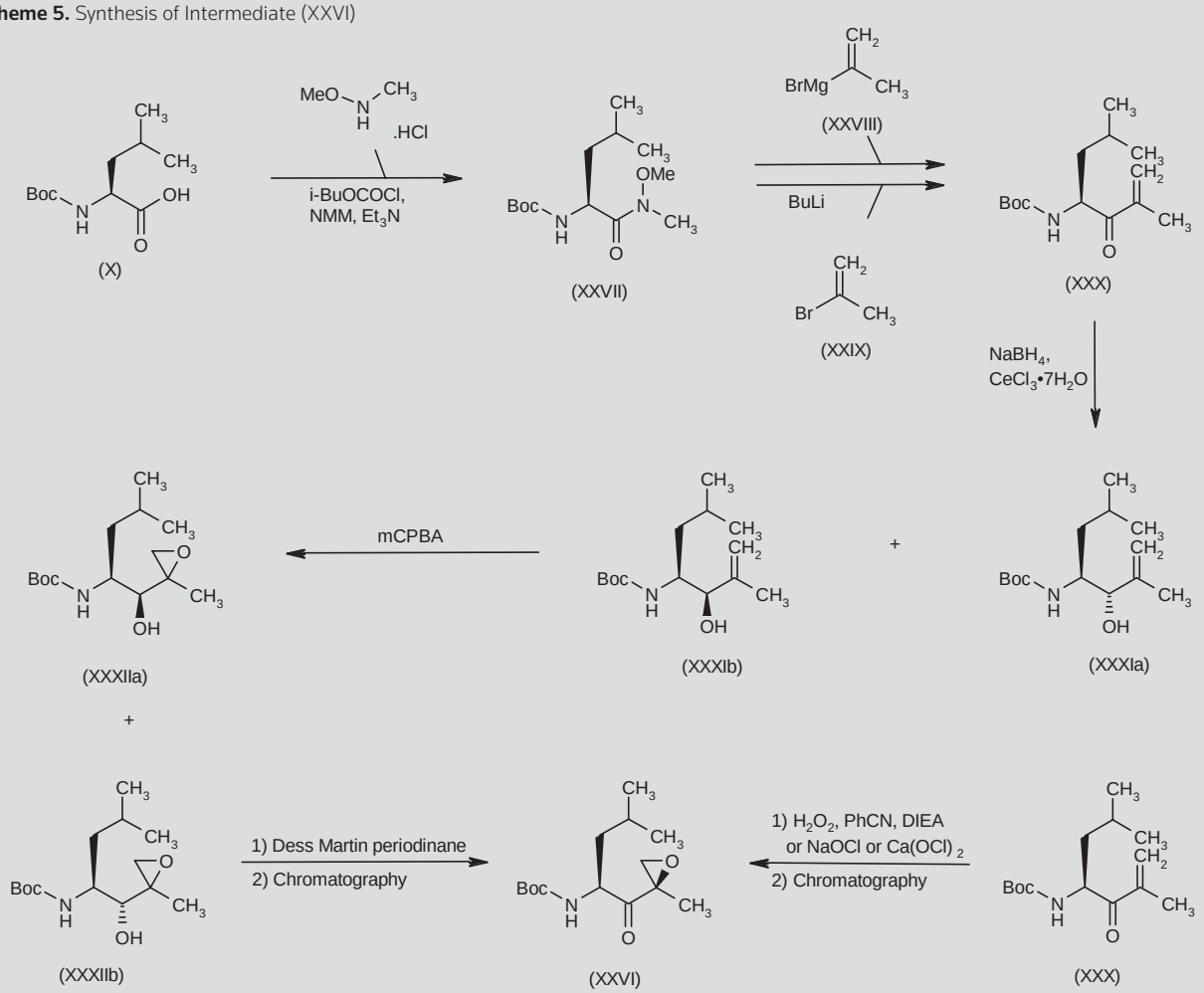


phoma (NHL). However, the development of resistance and toxicity among bortezomib-treated patients has led to the characterization of novel agents that elicit more selective proteasome targeting.

While the majority of cells express constitutive proteasomes, cells of the immune system and some types of hematological tumor cells express immunoproteasomes, which have three catalytic subunits that differ from those expressed in proteasomes (9). It has therefore been suggested that an immunoproteasome-specific inhibitor would have the potential advantage of selectively targeting proteasome function in hematological tumor cells, while sparing other tissues and limiting toxicity or adverse events that may be the result of constitutive proteasome inhibition in nonmalignant cells (10).

Proteolix is currently developing a novel, irreversible, epoxomicin-related proteasome inhibitor, carfilzomib (PR-171). This agent is presently undergoing phase I/II clinical evaluation in the U.S. Two open-label phase Ib trials have initiated recruitment: 1) to evaluate the safety and tolerability of carfilzomib in subjects with relapsed solid tumors and MM (11); and 2) to evaluate carfilzomib in combination with lenalidomide and dexamethasone for relapsed MM (12). In addition, a further four phase II open-label studies are also ongoing: 1) a multicenter study of carfilzomib to monitor the safety and efficacy of a maintenance therapy schedule for MM patients who previously completed a primary carfilzomib treatment study (13); 2) an assessment of the influence of renal impairment on carfilzomib in subjects with MM (14); 3) a trial to evaluate the overall response rate, safety and tolerability of carfilzomib in subjects with relapsed and refractory MM who have received prior treatment with bortezomib and either thalidomide or lenalidomide (15); and 4) a study of carfilzomib in patients with relapsed MM (16).

Scheme 5. Synthesis of Intermediate (XXVI)



PRECLINICAL PHARMACOLOGY

Initial in vitro studies demonstrated that carfilzomib inhibits the chymotrypsin-like activity of purified human 20S proteasome with a k_{inact}/K_i of 34,000 $M^{-1}s^{-1}$ and more than 300-fold selectivity over other proteasome catalytic activities. PR-171 also inhibited the chymotrypsin-like activity of the immunoproteasome (IC_{50} = 33 nmol/L). In addition, carfilzomib has been found to exhibit minimal activity against a broad and diverse panel of biochemical assays that includes 67 receptor/ligand and 37 enzyme assays (17, 18).

The ability of carfilzomib to inhibit the proteasome in intact cells has also been examined in HT-29 colorectal adenocarcinoma cells. A 1-h incubation with carfilzomib resulted in a concentration-dependent inhibition of all three proteasome catalytic activities, with the chymotrypsin-like activity exhibiting the greatest sensitivity (IC_{50} = 9 nmol/L). The caspase-like and trypsin-like activities were also inhibited (IC_{50} = 150-200 nmol/L). Despite irreversible binding of carfilzomib to the proteasome, the rate of recovery of proteasome activity in cultured cells (half-life approximately 24 h) was only moderately slower than that observed with the reversible agent bortezomib, suggesting that this is related to induction of mRNA transcription and de novo proteasome synthesis (19).

Assays conducted using the human MM cell line ANBL-6 have also shown that carfilzomib potently binds and inhibits the chymotrypsin-like proteasome (80% inhibition at concentrations > 10 nM) and immunoproteasome activities, specifically binding to the β5 constitutive proteasome and $\beta5_i$ immunoproteasome subunits. This has been shown to result in accumulation of ubiquitin–protein conjugates and proteasome substrates. These molecular observations also correlate with a concentration- and time-dependent inhibition of proliferation, ultimately leading to apoptosis, which was associated with activation of c-Jun-*N*-terminal kinase (JNK), mitochondrial membrane depolarization, release of cytochrome *c* and activation of both intrinsic and extrinsic caspase pathways, in that both caspase-8 and caspase-9 were potently induced (19-21).

An in vitro study evaluated the effects of carfilzomib on human MM cell survival, proteasome activity and drug resistance. It was shown that carfilzomib provides growth inhibition and apoptosis in the drug-sensitive human MM cell lines 8226/S, MM1, NCI-H929 and

U266, with IC_{50} values ranging from 9 to 21 nM, and activity against those resistant to melphalan, mitoxantrone and tipifarnib. Furthermore, in MM cells freshly isolated from patients carfilzomib (10-250 nM) induced apoptosis in nine of nine samples tested from both newly diagnosed and relapsed, refractory patients. Carfilzomib (1-100 nM) was also shown to overcome cell adhesion-mediated drug resistance in 8226/S and NCI-H929 cell lines and primary MM cells from six of nine patients (22).

The ability of carfilzomib to inhibit the proliferation of MM cells in a concentration- and time-dependent fashion was demonstrated in both interleukin-6 (IL-6)-dependent (ANBL-6 and KAS-6) and IL-6-independent (RPMI 8226 and U266) cell lines. It was noted that IL-6-dependent cells generally display greater sensitivity to carfilzomib-mediated effects compared to IL-6-independent cells (20).

The effects of bortezomib and carfilzomib on primary human acute myeloid leukemia (AML) cells were compared. Both drugs inhibited autocrine- and cytokine-dependent proliferation of primary AML blasts when tested at nanomolar levels (0.1-100 nmol/L). The antiproliferative effects of these drugs were shown to be independent of basal chymotrypsin-like proteasome activity, genetic abnormalities, morphological differentiation and CD34 expression when testing a large group of patients (N = 54). While both drugs enhanced apoptosis, the effects of carfilzomib could be detected at lower concentrations. The drugs had divergent effects on constitutive cytokine release from AML cells. Furthermore, both drugs caused a decrease in proliferation and viability when tested in combination with idarubicin or cytarabine (23).

Further investigations have also demonstrated efficacy for carfilzomib in purified $CD138^+$ plasma cells derived from patients. Continuous exposure of such cells to escalating concentrations of carfilzomib for 24 h, followed by measurement of chymotrypsin-like activity, revealed that the compound induces concentration-dependent proteasome inhibition and a significant reduction in cell viability. The antiproliferative effects of carfilzomib (100 nM pulse) were also shown to be greater than those of bortezomib in plasma cells from either bone marrow aspirates or peripheral blood of patients, some with chromosome 13 deletions. In addition, carfilzomib inhibited the proliferation of and induced apoptosis in purified samples from patients with diffuse large B-cell NHL, chronic lymphocytic leukemia (CLL) and AML, with a suggestion of enhanced activity compared to bortezomib. Carfilzomib overcame both primary and secondary resistance to bortezomib in both cell lines and clinical samples and acted synergistically with dexamethasone to enhance cell death (17, 19-21).

The extent to which bortezomib and carfilzomib inhibit nonproteasomal enzymes was investigated in an attempt to estimate the clinical toxicity profile. These in vitro studies demonstrated that, while carfilzomib does not appear to inhibit nonproteasomal targets, bortezomib does inhibit the nonproteasomal enzymes cathepsin G (IC_{50} = 0.3 μM), cathepsin A, dipeptidyl peptidase 4 and chymase (IC_{50} = 1.1 μM) (24).

Studies have also indicated the benefit of combining carfilzomib with a selective inhibitor of the cyclin-dependent kinases CDK4 and CDK6, PD-0332991. Targeting CDK4/6 with PD-0332991 in combination with carfilzomib led to complete eradication of myeloma cells ex vivo and this appeared to be due to synergistic halting of cell proliferation and apoptosis, which is likely to eliminate cell cycle reentry and the generation of resistant cells (25).

Repeated administration for up to 5 consecutive days was well tolerated even at doses resulting in peak inhibition of the proteasome chymotrypsin-like activity in excess of 80% in blood and most other tissues. In BNX (beige/nude/xid) mice bearing established human tumor xenografts derived from three tumor cell lines (colorectal adenocarcinoma HT-29, B-cell lymphoma RL and Burkitt's lymphoma HS-Sultan) the antitumor efficacy of carfilzomib was dose- and schedule-dependent; however, 5 mg/kg i.v. on 2 consecutive days weekly was found to be more effective than 1 mg/kg bortezomib (i.v. biweekly day 1/day 4) (18, 26).

PHARMACOKINETICS AND METABOLISM

The in vitro metabolism of carfilzomib was assessed following incubation with liver microsomes from rats, monkeys and humans, in blood from rats and humans and in homogenates of rat lung, heart and kidney. These studies confirmed that carfilzomib is rapidly metabolized in liver microsomes from all species tested (27).

An in vivo study characterized the pharmacokinetics of carfilzomib following a single i.v. dose to rats and monkeys. Analysis of plasma samples demonstrated that carfilzomib is rapidly cleared from the plasma compartment following i.v. administration. In experimental animals there was a dose-dependent increase in the AUC. The terminal plasma half-life was reported to be 15 and 7.2 min, respectively, in rats and monkeys and the systemic clearance was 520 mL/min/kg in rats and 236 mL/min/kg in monkeys, suggesting that clearance of carfilzomib occurs extrahepatically. The predominant metabolites detected in rats and monkeys were products of peptidase and epoxide hydrolase activity (peptide fragments and carfilzomib diol, respectively), and therefore cytochrome P450-mediated metabolism does not appear to play a major role in the clearance of carfilzomib (27, 28).

The pharmacokinetic characteristics of carfilzomib were investigated further in rats. The compound (1 mL/kg at 2, 4.5 and 9 mg/kg) was rapidly cleared from the plasma compartment following i.v. administration. Noncompartmental analysis of carfilzomib pharmacokinetics confirmed an average terminal plasma half-life of approximately 15 min, with estimated steady-state volume of distribution values suggesting extensive penetration into peripheral tissues (18, 26).

Mass balance and tissue distribution have also been investigated in rats using [^{3}H]-carfilzomib. Radioactivity was widely distributed following a single i.v. bolus dose and reached maximum concentrations at times ranging from 0.5 to 24 h in different tissues. At 168 h post-dose, more than 44% of the administered radioactivity remained in tissues. Excretion via urine and feces accounted for 14% and 18%, respectively, of administered radiation (22).

CLINICAL STUDIES

A clinical study assessed the effects of carfilzomib in patients with MM, NHL, Hodgkin's disease (HD) or Waldenström's macroglobu-

linemia (WM) on two different dose-intensive schedules (N = 54). In PX-171-001 carfilzomib was administered on a 2-week cycle, given once daily for 5 consecutive days with 9 days of rest; in PX-171-002 carfilzomib was administered on a 4-week cycle, given once daily for 2 consecutive days for 3 weeks with 12 days of rest. Minimal effective doses (MEDs) on PX-171-001 and -002 were reported as 11 and 15 mg/m^2, respectively. A partial response (PR) was observed in one MM patient and one WM patient had a minimal response (MR) in the PX-171-001 study. PRs were reported for three of eight MM patients on the PX-171-002 protocol. Stable disease (SD) of > 6 months was demonstrated in a total of six patients and symptomatic improvement was seen in patients on both protocols. Carfilzomib was well tolerated at doses at and above the MED, with no incidence of painful peripheral neuropathy in either study. No dose-limiting toxicities (DLTs) were recorded in patients in PX-171-001; one DLT (grade 4 anemia and thrombocytopenia) was observed at 27 mg/m^2 in PX-171-002. Analysis of whole blood samples confirmed that proteasome inhibition at the highest dose levels exceeded 80% 1 h after the first dose (29-32).

An open-label, multicenter study (PX-171-003) assessed carfilzomib in MM patients who had relapsed from at least two prior therapies (bortezomib and either thalidomide or lenalidomide) and who were refractory to other treatments (defined as disease progression on or within 60 days of last therapy or < 25% response to the last therapy). Patients received carfilzomib 20 mg/m^2 i.v. on days 1, 2, 8, 9, 15 and 16 every 28 days for up to 12 cycles. Oral dexamethasone (4 mg) was given prior to each dose during cycle 1. Current data from 39 of the 46 patients enrolled who completed a median of three carfilzomib cycles (evaluated by the International Uniform Response Criteria for MM) indicate that 13% had a PR, 13% had an MR (for an overall clinical benefit response of 26%), 41% had SD and 28% had disease progression. Among the 26 patients with SD, MR or PR, all of whom were bortezomib-refractory, there was one PR lasting 211 days and 4 MRs lasting 100-332 days. Time to response was rapid, frequently occurring in the first cycle. Carfilzomib was generally well tolerated, with the most common adverse events reported as fatigue (65%), nausea (37%), upper respiratory infection (37%) and diarrhea (33%). Grade 3 or 4 anemia occurred in 33% of the patients, grade 3 or 4 thrombocytopenia occurred in 23% and grade 3 or 4 neutropenia occurred in 4.3%. Four patients had acute renal failure and two of these patients also had possible tumor lysis. In other patients, changes in creatinine clearance levels were transient and reversible. Grade 1 or 2 peripheral neuropathy was evident in 78% of patients at baseline, although exacerbation with carfilzomib was rare and no study discontinuations or dose reductions were seen due to neurotoxicity (33).

In PX-171-004, an open-label, multicenter study, single-agent carfilzomib was given to patients with relapsed MM who had been treated with no more than three prior therapies. In this trial, 31 patients were stratified into bortezomib-exposed (17 patients) or bortezomib-naïve (14 patients) cohorts. Patients received carfilzomib 20 mg/m^2 i.v. on days 1, 2, 8, 9, 15 and 16 every 28 days for up to 12 cycles. Oral dexamethasone (4 mg) was given prior to each dose during cycle 1. In bortezomib-naïve patients carfilzomib achieved an overall response rate (ORR) of 57%, with a median duration of response (DOR) of 8.6 months. At the time of reporting, 7 patients (50%) remained progression-free and 3 patients had completed 12 cycles. The median follow-up was 10 months and the median time to progression (TTP) had not been reached. For the bortezomib-exposed group carfilzomib achieved an ORR of 18%; the median DOR had not yet been reached (> 8.5 months; range: > 1 day to > 8.5 months). Seven patients (41%) were reported to be progression-free and 3 patients had completed 12 cycles. Median follow-up and TTP were 9.2 and 8.9 months, respectively. Hematological adverse events included neutropenia in 32%, with grade 3 or 4 neutropenia in 10%, anemia in 29% (6.5% grade 3/4) and thrombocytopenia in 23% (6.5% grade 3/4). The only grade 3 or 4 nonhematological adverse event was dyspnea, which occurred in 6.5% of patients. One patient in the bortezomib-naïve group had confirmed tumor lysis syndrome and one had a possible case. These cases led to a protocol amendment in April 2008, with prophylaxis guidelines for hydration and allopurinol; since the change, more than 80 patients have been enrolled and no further cases of the syndrome have been seen. Although 73% of patients had a history of drug- or disease-related peripheral neuropathy and 32% had neuropathy at baseline, there was only one case of grade 1 neuropathy and no grade 3 or 4 neuropathy reported (34, 35).

Preliminary data from an ongoing phase Ib dose-escalating trial of carfilzomib (15-20 mg/m^2 i.v. on days 1, 2, 8, 9, 15 and 16) in combination with lenalidomide (10-20 mg p.o. on days 1-21) and low-dose dexamethasone (40 mg p.o. on days 1, 8, 15 and 22) in relapsed/refractory MM patients who failed one to three prior therapies including prior lenalidomide or bortezomib were presented. The combination of carfilzomib and lenalidomide was reported to be well tolerated and encouraging response rates were observed, with 61% of 18 evaluable patients achieving PRs or MRs, including 3 very good PRs. Notably, responses occurred in the first 28-day cycle of treatment and initial responses improved with continued therapy. A maximum tolerated dose had not yet been established and dose escalation continues in this study (36).

SOURCE

Proteolix, Inc. (US).

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