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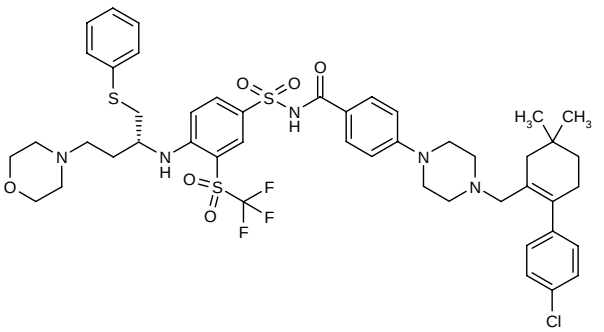
# ABT-263

*Bcl-2 Inhibitor*
*Apoptosis Inducer*
*Oncolytic*

*N*-[4-[4-[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexenylmethyl]piperazin-1-yl]benzoyl]-4-[3-(4-morpholinyl)-1(*R*)-(phenylsulfanylmethyl)propylamino]-3-(trifluoromethylsulfonyl)benzenesulfonamide

InChI=1/C47H55ClF3N5O6S3/c1-46(2)20-18-42(34-8-12-37(48)13-9-34)36(31-46)32-55-22-24-56(25-23-55)39-14-10-35(11-15-39)45(57)53-65(60,61)41-16-17-43(44(30-41)64(58,59)47(49,50)51)52-38(19-21-54-26-28-62-29-27-54)33-63-40-6-4-3-5-7-40/h3-17,30,38,52H,18-29,31-33H2,1-2H3,(H,53,57)/t38-/m1/s1



$C_{47}H_{55}ClF_3N_5O_6S_3$
Mol wt: 974.613

CAS: 923564-51-6

EN: 474044

**Abstract**

Antiapoptotic Bcl-2 family proteins are overexpressed in many types of tumors and are believed to be associated with tumor formation, tumor growth and drug resistance. ABT-263 is a small-molecule inhibitor of Bcl-2 proteins designed to restore apoptosis. Like its predecessor ABT-737, ABT-263 exhibits strong binding to the antiapoptotic proteins Bcl-2, Bcl-xL and Bcl-w, with subnanomolar affinity. In vivo, ABT-263 induced dose-dependent tumor regression. Treatment with ABT-263 100 mg/kg/day for 21 consecutive days induced complete tumor regression (100% overall response rate [ORR]) in small cell lung cancer (SCLC) and acute lymphoblastic leukemia (ALL) xenograft models. In the NCI-H146 SCLC xenograft model, the efficacy of ABT-263 at or above 100 mg/kg/day was superior to that of all other cytotoxic agents tested, including paclitaxel, vincristine, carboplatin, cisplatin, cyclophosphamide and etoposide. A series of phase I/IIa clinical trials evaluating the safety, pharmacokinetics and efficacy of ABT-263 in patients with relapsed or refractory lymphoid malignancies, relapsed or refractory chronic lymphocytic leukemia (CML), SCLC or other nonhematological malignancies are ongoing.

## Synthesis

Ring opening of 3(*R*)-(benzyloxycarbonylamino)-γ-butyrolactone (I) with morpholine (II) in dioxane at 65 °C provides the morpholide (III), which is condensed with diphenyl disulfide in the presence of $Bu_3P$ in hot toluene, yielding the phenyl thioether (IV). Then, the benzyloxycarbonyl protecting group is removed with 30% HBr in AcOH, and the resulting deprotected amino-amide (V) is reduced to diamine (VI) using a solution of borane in tetrahydrofuran (1). Subsequent coupling of diamine (VI) with 4-fluoro-3-(trifluoromethylsulfonyl)benzenesulfonamide (VII) in the presence of DIEA in THF or DMSO gives the sulfanilamide (VIII) (1-3), which is finally acylated with the piperazinylbenzoic acid derivative (IX) by means of EDC and DMAP in $CH_2Cl_2$ (1-4). Scheme 1.

The precursor 4-fluoro-3-(trifluoromethylsulfonyl)benzenesulfonamide (VII) can be prepared by the following methods:

Condensation of 2-fluorobenzenethiol (X) with iodotrifluoromethane in the presence of triethylamine and methyl viologen (1,1'-dimethyl-4,4'-bipyridinium dichloride) yields the trifluoromethyl sulfide (XI), which is oxidized to sulfone (XII) by means of $NaIO_4$ and catalytic $RuCl_3$ (1, 2). Subsequent chlorosulfonation of (XII) with $ClSO_3H$ affords the acid chloride (XIII), which is finally converted to the target sulfonamide (VII) upon quenching with ammonium hydroxide in cold isopropanol (1-3). Scheme 2.

In an improved method, treatment of bis(*o*-nitrophenyl)disulfide (XIV) with potassium trifluoroacetate in sulfolane at 180-230 °C provides the trifluoromethyl sulfide (XV), which is oxidized to the corresponding sulfone derivative (XVI) using periodic acid and a catalytic amount of $CrO_3$. Finally, compound (XIV) is submitted to nitro group displacement with dried potassium fluoride

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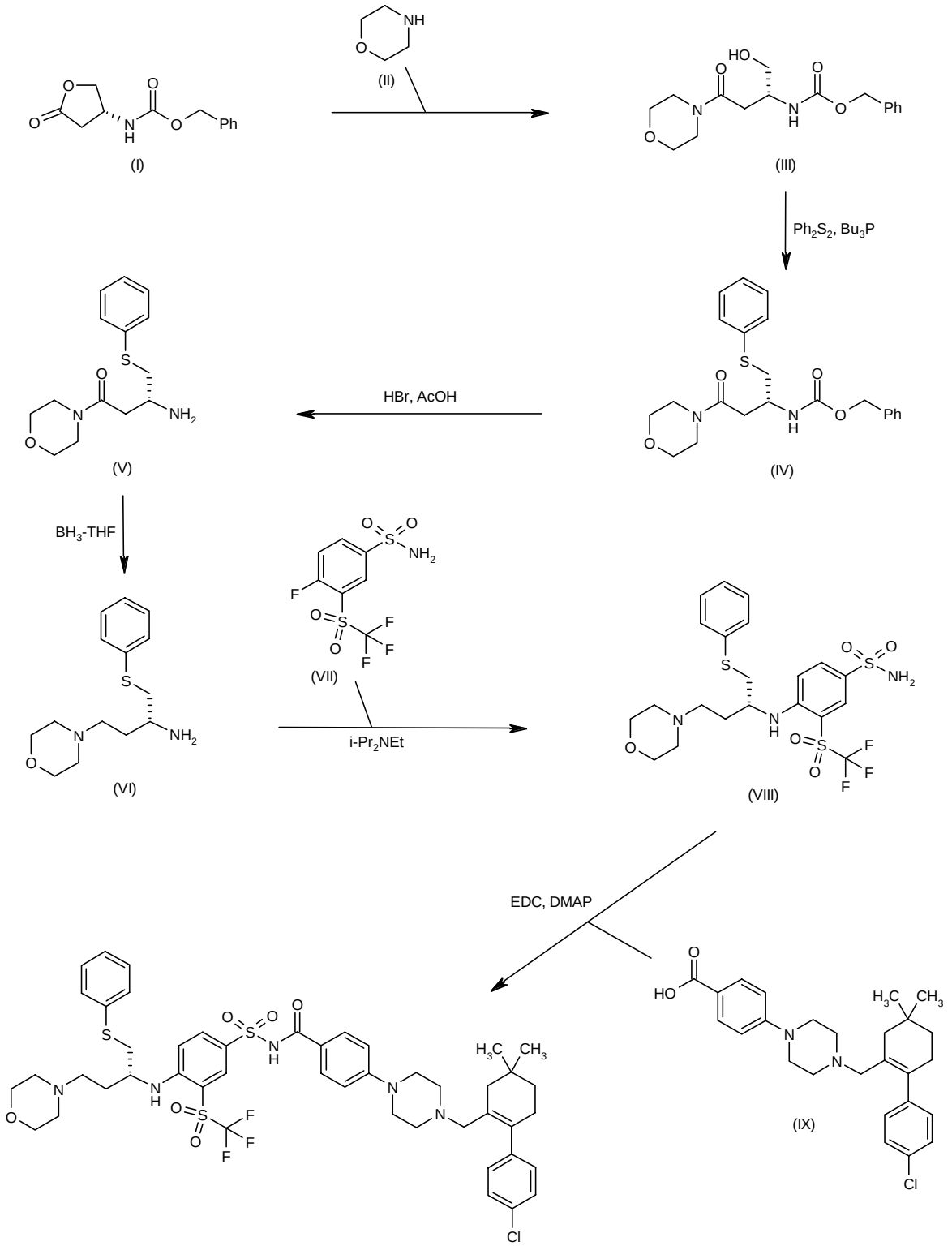
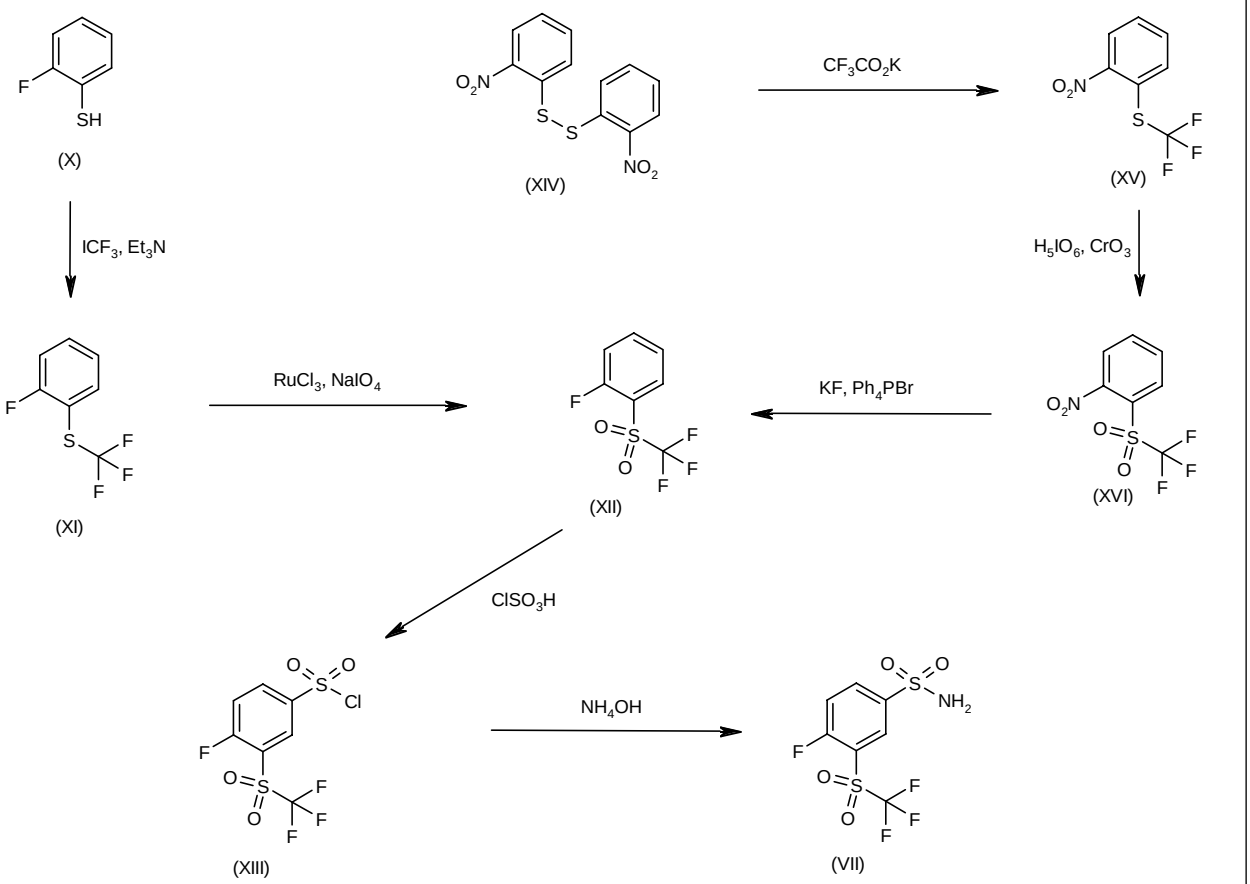
**Scheme 1: Synthesis of ABT-263**



**Scheme 2: Synthesis of Intermediate (VII)**




and tetraphenylphosphonium bromide in hot DMSO (3). Scheme 2.

The building block (IX) can be obtained by several related methods:

Reaction of 4,4-dimethylcyclohexanone (XVII) with DMF and $PBr_3$ under Vilsmeier formylation conditions provides the carbaldehyde (XVIII), which is subjected to reductive amination with 4-piperazinobenzoic acid ethyl ester (XIX) and $NaBH_3CN$ resulting in the cyclohexenylmethyl piperazine (XX). Subsequent Suzuki coupling of compound (XX) with 4-chlorophenylboronic acid (XXI) followed by ethyl ester hydrolysis of the resultant adduct (XXII) leads to the target intermediate (IX) (1). Scheme 3.

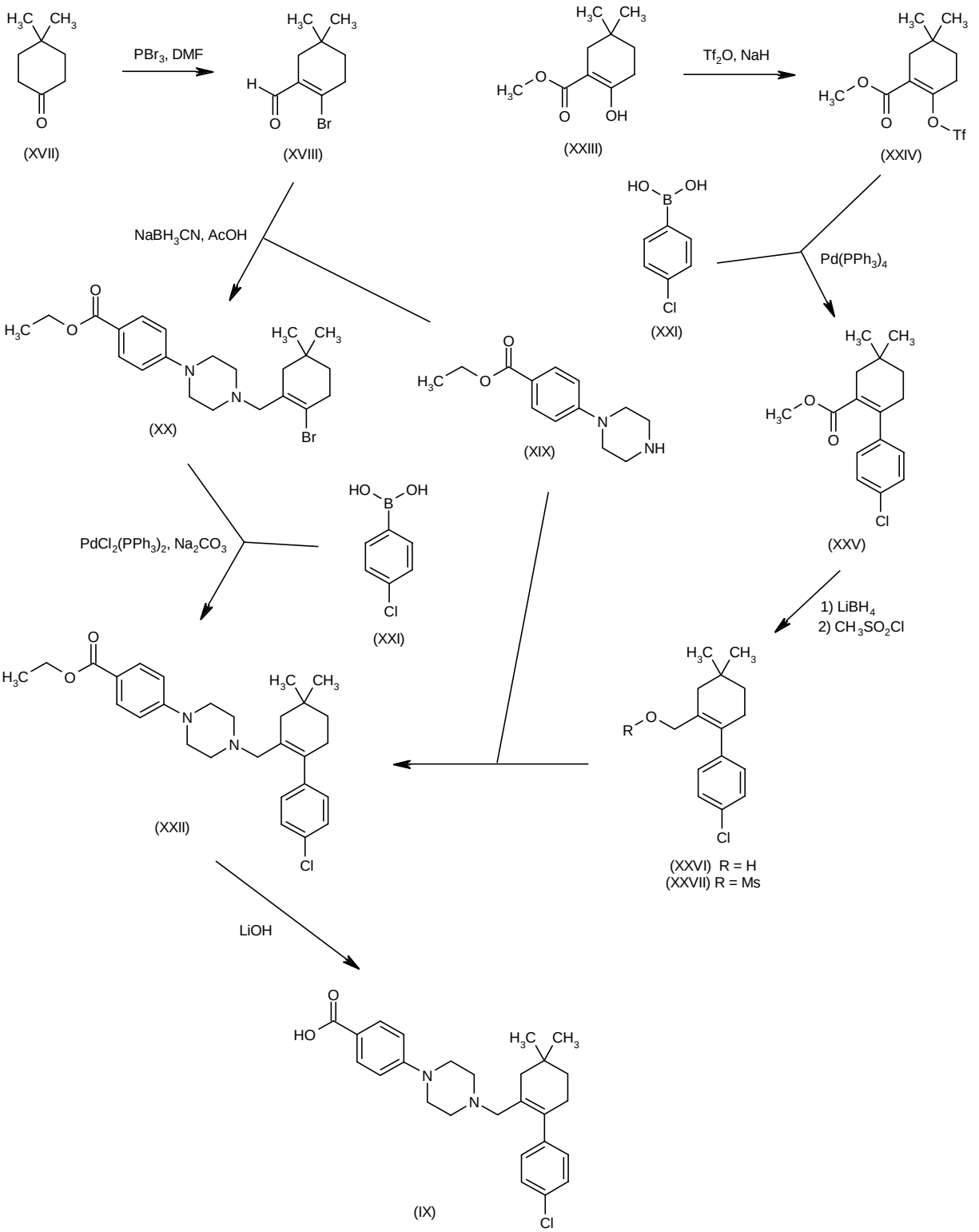
In a related procedure, 2-hydroxy-5,5-dimethyl-1-cyclohexenecarboxylic acid methyl ester (XXIII) is converted to the corresponding triflate (XXIV), which undergoes Suzuki coupling with boronic acid (XXI) to yield the 2-arylcyclohexenecarboxylate derivative (XXV). After reduction of ester (XXV) with $LiBH_4$, the resulting primary alcohol (XXVI) is converted to the corresponding mesylate (XXVII) with methanesulfonyl chloride, and then condensed with the piperazine derivative (XIX) followed by ethyl ester hydrolysis to yield the key precursor (IX) (4). Scheme 3.

In a further method, Mannich reaction of 4,4-dimethylcyclohexanone (XVII) with *tert*-butyl 4-piperazinobenzoate (XXVIII) and paraformaldehyde by means of HCl in *tert*-butanol affords the piperazinylmethyl-cyclohexanone (XXIX), which is further condensed with 4-chlorophenylmagnesium bromide (XXX), yielding carbinol (XXXI). Simultaneous dehydration of alcohol (XXXI) and cleavage of the *tert*-butyl ester in refluxing 6 M HCl gives carboxylic acid (IX) (3). Scheme 4.

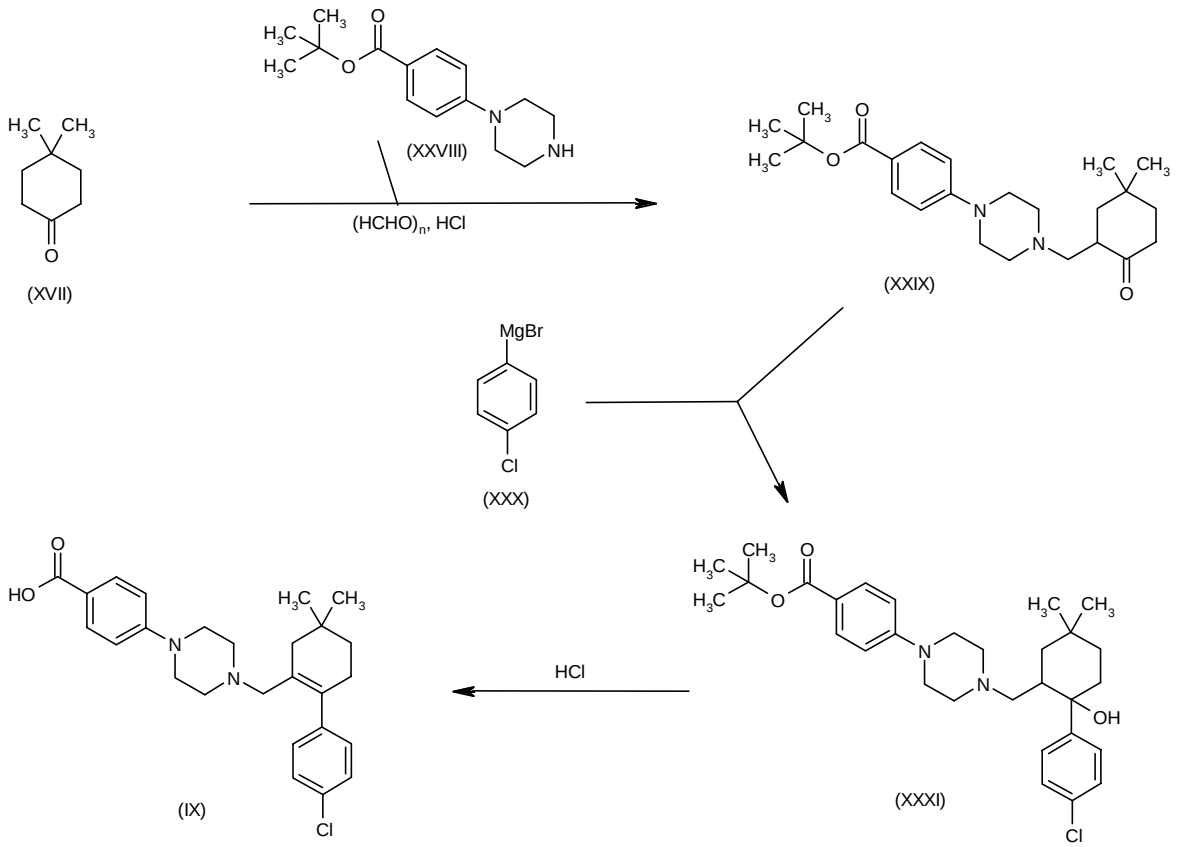
## Background

Apoptosis (programmed cell death) is the body's natural way of getting rid of abnormal or unneeded cells. Disruption of the regulation of apoptosis can cause various diseases, including cancer. Bcl-2 family proteins are central regulators of apoptosis. Characterized by the presence of one or more of the four Bcl-2 homology (BH) domains (BH1, BH2, BH3 and BH4), Bcl-2 family proteins can be divided into three subfamilies according to the

**Scheme 3: Synthesis of Intermediate (IX)**



**Scheme 4: Alternative synthesis of Intermediate (IX)**




number of BH homology domains that the proteins conserve. Members of the first subfamily, including BAX, BAK and BOK, contain 3 BH domains (BH1, BH2 and BH3). These proteins are required for apoptosis, and they function by triggering the release of caspases from death antagonists and by inducing the release of mitochondrial apoptogenic factors such as cytochrome *c* into the cytoplasm. Conserving all 4 BH domains, members of the second subfamily are antiapoptotic. Members of this subfamily include Bcl-2, Bcl-xL, Bcl-w, BFL-1, Mcl-1 and A1. These antiapoptotic (also known as prosurvival) proteins function by either sequestering apoptosome or preventing the release of mitochondrial apoptogenic factors into the cytoplasm. Furthermore, Bcl-2 and Bcl-xL prevent oligomerization of BAX and BAK by directly binding to these proteins. Members of the third subfamily of the Bcl-2 family, including BID, BIM, BAD, BIK, BMF, HRK, NOXA and BBC3 (PUMA), contain only a BH3 domain, and they are therefore called BH3-only proteins. Members of this subfamily are proapoptotic. These proteins initiate their activities by direct interacting with Bcl-2 or its functional homologues by inserting their BH3 domain into a hydrophobic groove (BH3 groove) on the surface of the antiapoptotic proteins (5-10).

Antiapoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-xL are overexpressed in many types of cancers, including B-cell lymphomas and small cell lung cancer (SCLC), and they are believed to contribute to tumor initiation, progression and drug resistance. Antiapoptotic proteins are therefore attractive targets for antitumor therapy. ABT-737, a BH3-only mimetic, is a first-generation small-molecule inhibitor of antiapoptotic Bcl-2 family proteins. The agent exhibits high-affinity binding to Bcl-2, Bcl-xL and Bcl-w. In early studies, ABT-737 demonstrated potent antitumor activity against human SCLC and lymphoid tumor cell lines. The agent also effectively induced apoptosis in patient-derived lymphoma and chronic lymphocytic leukemia (CLL). Its poor solubility, however, limited its further clinical use (11-20).

ABT-263, also a BH3-only protein mimetic, is a second-generation antiapoptotic Bcl-2 family protein inhibitor. The compound was designed by modifying the structure of ABT-737. ABT-263 and ABT-737 exhibit very similar binding profiles for Bcl-2 family proteins, both exhibiting strong binding to Bcl-2, Bcl-xL and Bcl-w but weaker binding to Mcl-1 and A1. Unlike ABT-737, which is a parenteral agent, ABT-263 is orally bioavailable (2, 4, 21, 22). A series of phase I/IIa clinical trials evaluating

ABT-263 as a single agent for the treatment of relapsed or refractory CLL, relapsed or refractory lymphoid malignancies and advanced SCLC are ongoing (23-27).

## Preclinical Pharmacology

As indicated above, ABT-263 exhibits strong binding to Bcl-2, Bcl-xL and Bcl-w ($K_i \leq 1$ nmol/l) but much weaker binding to Mcl-1 ($K_i$ ~ 0.5 μmol/l) and A1 ($K_i$ ~ 0.3 μmol/l). The enantiomer of ABT-263 exhibited greatly reduced binding affinity and was markedly less active in a variety of assays (22).

The mechanisms of cytotoxicity of ABT-263 were evaluated in an IL-3-dependent prolymphocytic FL5.12 murine cell line. The presence of the caspase inhibitor ZVAD markedly weakened the ability of ABT-263 to kill FL5.12-Bcl-2 or FL5.12-Bcl-xL cells, indicating that cell killing is caspase-dependent. Co-immunoprecipitation studies demonstrated that ABT-263 concentration-dependently disrupted BIM:Bcl-xL interactions in FL5.12-Bcl-xL cells (within 2 h posttreatment) and BIM:Bcl-2 interactions in FL5.12-Bcl-2 cells, indicating that it restores IL-3-dependent cell death by disrupting the Bcl-2 family protein–protein interactions. Similar results were observed in other mammalian systems (22).

The ability of ABT-263 to directly induce apoptosis was evaluated in the SCLC cell line NCI-H146, which depends on Bcl-2 for survival. ABT-263 induced BAX translocation and cytochrome *c* release, and led to subsequent apoptosis within 2 h after treatment. The enantiomer, however, failed to induce similar responses (22, 28).

ABT-263 as a single agent exhibited cytotoxicity against SCLC and hematological malignancies but not other types of cancer cells. In each of the panels of cell lines derived from SCLC (N = 22) and hematological malignancies (N = 23), ABT-263 exhibited a range of potency. Thirty-two percent of the SCLC cell lines and 48% of the hematological cell lines were highly sensitive ($EC_{50}$ < 1 μmol/l) to ABT-263. The enantiomer was again less active (> 20-fold) in these sensitive cells (22, 28). Similar results were obtained both in vitro and in vivo using the Pediatric Preclinical Testing Program (PPTP) panel of cell lines and xenograft models (29).

The in vivo activity of ABT-263 was evaluated in xenograft models established using some of the sensitive SCLC and hematological cell lines. Oral administration of ABT-263 once daily at 100 mg/kg for 21 consecutive days induced a rapid and complete tumor response (100% CR), which was defined as regression of tumor beyond the level of detection using palpation. In all animals bearing SCLC NCI-H889 or acute lymphocytic leukemia (ALL) RS4;11 xenografts, regression lasted for several weeks after the end of treatment. Similar treatment led to CR in 60% and partial response (PR), which was defined as regression of an established tumor by ≥ 50% from its starting volume, in 40% of mice bearing SCLC NCI-H146 tumors. In this model, gradual tumor rebound was observed several weeks after the end of treatment. ABT-263 at lower dose levels induced fewer CR and PR in the animals: administration of 50 mg/kg ABT-263 for 21 consecutive days induced CR in 22% and PR in 44% of the animals, and administration of 25 mg/kg for 21 days induced modest tumor growth inhibition but no tumor regression. ABT-263 was associated with < 5% weight loss at all doses tested. Based on these results, 50 mg/kg/day was defined as the minimum effective dose (MED) in the NCI-H146 xenograft model, which is resistant to treatment with paclitaxel. As observed for ABT-737, treatment with ABT-263 induced a rapid but reversible decrease in circulating platelets. For example, a single dose (100 mg/kg) of ABT-263 reduced circulating platelet counts by ~90% from baseline in CF-1 mice. However, the platelet counts returned to baseline values within 72 h. Dose escalation led to a further decrease in platelets, but multiple daily dosing at the same level did not significantly further reduce platelet counts after the first dose (22, 28).

The effect of dose and dosing regimen of ABT-263 on its antitumor efficacy was further evaluated using the NCI-H146 model. Doses (25, 50, 100, 200 and 300 mg/kg/day) given on a continuous basis were examined. All the doses evaluated induced significant tumor growth inhibition ($P < 0.01$). Doses of 100 mg/kg/day and above produced 100% overall response rate (ORR), with CR observed in the majority of the animals (60% for 100 mg/kg/day, 90% for 200 mg/kg/day and 87% for 300 mg/kg/day). ABT-263 was well tolerated at doses of 100 mg/kg/day and below, but 1 death occurred in the 300 mg/kg/day group for unknown reasons. Both continuous and intermittent dosing of ABT-263 led to significant inhibition of tumor growth, but continuous dosing showed a stronger effect on tumor growth delay and resulted in a higher percentage of tumor regressions. Continuous dosing for 21 days produced the most robust efficacy, with 100% ORR. Once-daily dosing at 100 mg/kg and twice-daily dosing at 100 mg/kg/day (50 mg/kg/dose) were equally effective (28).

Comparison of the antitumor activity of ABT-263 with other cytotoxic agents, including paclitaxel, vincristine, carboplatin, cisplatin, cyclophosphamide and etoposide, was conducted using the NCI-H146 xenograft model. Paclitaxel administered at 30 mg/kg/day (every 4 days x 3) produced PR in 4 of 6 (67%) tumors and CR in 2 of 6 (33%) tumors. Vincristine at 0.5 mg/kg/day (every 4 days x 4) induced PR in 5 of 9 (56%) tumors and CR in 1 of 9 (11%) tumors. Treatment with carboplatin, cisplatin, cyclophosphamide and etoposide did not lead to tumor regression. The efficacy of ABT-263 at doses of 100 mg/kg/day or above (100% ORR) was superior to that of all the cytotoxic agents tested. Treatment with ABT-263 at 100 mg/kg/day was able to reduce NCI-H146 tumors of ~1000 $mm^3$ by > 80% from baseline. Similar reductions were observed for docetaxel at a dose of 30 mg/kg i.v. every 7 days x 2, although this dose was not tolerated as it caused > 20% weight loss after the second dose (28).

In theory, combining ABT-263 with agents that down-regulate the apoptosis pathway could broaden the efficacy of ABT-263. Recent studies demonstrated that ABT-

263 combined with bortezomib showed potent in vivo activity in the ABT-263-resistant OPM-2 flank xenograft model of multiple myeloma that expresses high levels of Mcl-1 (downregulation). At its maximum tolerated dose (MTD; 1 mg/kg/day i.v. x 3), bortezomib inhibited tumor growth by 79%, with no CRs. However, bortezomib combined with ABT-263 resulted in 95% tumor growth inhibition and 40% CRs (22).

The in vivo activity of ABT-263 combined with other therapeutic agents such as rituximab was also investigated in B-cell lymphoma xenograft models. ABT-263 (100 mg/kg/day p.o. for 17 days) produced 44% tumor growth inhibition. A single dose (10 mg/kg) of rituximab induced 84% tumor growth inhibition. Combination of ABT-263 and rituximab produced 80% ORR (70% CR and 10% PR), whereas neither ABT-263 nor rituximab alone resulted in sustained tumor regression. Compared with rituximab alone, combination of ABT-263 and rituximab also improved tumor growth delay (> 700% for the combination vs. 300% for rituximab), determined by the percent increase in life span (22).

The activity of ABT-263 combined with the R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine and prednisolone) regimen was studied in the GRANTA-519 mantle cell lymphoma flank xenograft model. Combination of ABT-263 and R-CHOP led to sustained tumor regression and complete tumor response in all animals tested, whereas ABT-263 at 100 mg/kg alone induced only 40% tumor growth inhibition and R-CHOP inhibited tumor growth by 68%, with 20% CR (22).

## Pharmacokinetics and Metabolism

The pharmacokinetics of ABT-263 were first evaluated in non-tumor-bearing mice administered the compound orally once daily for 3 days. Both maximum plasma concentration ($C_{max}$) and area under the plasma concentration curve (AUC) were dose-proportional. The $C_{max}$ and AUC of ABT-263 at 100 mg/kg, which induced 100% ORR, were 7.7 μmol/l and 90 μmol/l.h, respectively. The plasma $C_{max}$ and AUC of ABT-263 at 50 mg/kg, which was defined as the MED (~ 66% ORR and ~ 75% tumor growth inhibition), were 5.4 μmol/l and 54 μmol/l.h, respectively (22, 28).

In mice, rats, dogs and monkeys, ABT-263 showed a low volume of distribution and plasma clearance, with plasma elimination half-lives ranging from 4.6 to 8.4 h following i.v. doses. In all species tested, the bioavailability of ABT-263 after oral gavage was ~20%. Because of its low aqueous solubility, ABT-263 displayed prolonged oral absorption. When administered in lipid-based formulations, ABT-263 showed enhanced absorption and bioavailability (near 50%), with an oral elimination half-life of 8.9 h in dogs (22).

The pharmacokinetic profile of ABT-263 at fixed daily doses in patients with relapsed or refractory SCLC or other solid tumors was evaluated. The exposure of ABT-263 was dose-proportional. The average peak time of exposure was 8 h and the half-life of ABT-263 ranged from 14 to 20 h. During the first 14 days of dosing, ABT-263 exhibited time-invariant pharmacokinetic properties. The AUC of ABT-263 was not affected by body weight or body surface are (30).

The pharmacokinetics of ABT-263 were also evaluated in patients with relapsed or refractory lymphoid malignancies. In patients with relapsed CLL or small lymphocytic lymphoma (SLL), the pharmacokinetic profile of ABT-263 was linear at doses of 10-440 mg, with corresponding half-lives ranging from 14 to 20 h (31).

## Safety

Thrombocytopenia has been seen in several species following administration of ABT-263. In vitro studies in isolated dog and human platelets indicated that the reduction in platelet viability is an apoptotic-like response mediated by inhibition of antiapoptotic Bcl-2 family members (32). In vivo studies in CF-1 and inbred SCID beige mice showed a rapid, dose-dependent decrease in circulating platelets, with nadir at about 6 h after single oral doses of 100 mg/kg, which returned to normal by 72 h; platelet levels did not further decline with multiple doses. Reduced platelet count was correlated with increased bleeding time, but no significant bleeding or weight loss was seen in mice treated for up to 21 days at 100 mg/kg/day (33). Finally, dogs were given single i.v. or oral doses of ABT-263, which induced a rapid, dose-dependent reduction in platelet counts with nadir at about 6 h, returning to normal within several days. ABT-263 appeared to affect circulating platelets rather than platelet production in the bone marrow (34).

## Clinical Studies

The safety of ABT-263 in patients with relapsed or refractory lymphoid malignancies is being studied in an ongoing phase I/IIa clinical trial. Thirty patients enrolled in the study were assigned to doses of 10, 20, 40, 80, 160, 225 and 315 mg. In the 21-day treatment cycles, patients received ABT-263 orally every day for the first 14 days and were then off ABT-263 for 7 days. The dose was doubled in each cohort until grade 3 toxicity occurred, after which the dose was then escalated in 40% increments. One grade 3 toxicity was observed during cycle 1 at doses of 160 and 315 mg. Early results of the study showed that 2 patients with bulky CLL in the 40-mg cohort achieved 99% tumor reduction after cycle 8; 1 patient with bulky CLL/SLL in the 160-mg cohort experienced 75% tumor reduction at cycle 4; 1 patient with follicular lymphoma in the 80-mg cohort had 20% tumor reduction after cycle 7; and 1 patient with NK/T-cell lymphoma in the 315-mg cohort achieved a 75% reduction at cycle 2. Results of the study also indicated that ABT-263 reduced platelet levels in a dose-dependent manner. Grade 3 thrombocytopenia (without bleeding) was observed in 5 patients in the first cycle (25, 35-37).

To study the safety and pharmacokinetics of ABT-263 in patients with relapsed or refractory CLL, a nonrandom-

ized, open-label, uncontrolled safety and efficacy study (phase I/IIa clinical trial) was initiated. The purpose of the phase I portion of the study is to evaluate the pharmacokinetic profile and safety of ABT-263 on two different dosing schedules (14 days on therapy and 7 days off therapy during the 21-day cycles) with the objective of defining the dose-limiting toxicity (DLT) and MTD. The purpose of the second part of the study is to obtain additional safety information and a preliminary assessment of efficacy (23). Early results of this trial showed 2 confirmed PR (75% and 99% CT regression of adenopathy), 2 cases of stable disease (33% and 36% nodal regression), 5 patients with a > 50% decrease in circulating lymphocytes and 9 patients with progressive disease (4 on 10 mg, 2 on 20 mg and 3 on other doses). ABT-263 was associated with antitumor activity in 9 of the 23 (39%) treated CLL or SLL patients. Dose-dependent reductions in circulating platelet counts were also observed in the study (31).

The safety, pharmacokinetics and efficacy of ABT-263 in patients with SCLC or other nonhematological malignancies are also being evaluated in a phase I/IIa trial. In this nonrandomized, open-label study, patients are on therapy for 14 days then off therapy for 7 days in each of the 21-day cycles (24). Mechanism-based, dose-related thrombocytopenia was observed in the 30 patients enrolled in the ongoing study. The platelet nadir was observed early in the cycle. At 325 mg, the median, minimum and maximum platelet nadirs were 30%, 10% and 40% of the baseline value, respectively, and at 425 mg, the respective values were 19%, 9% and 31%. Stable disease was observed in 6 patients at cycle 2 assessment: 1 patient (SCLC) receiving 10 mg ABT-263, 3 patients (1 carcinoid and 2 SCLC) receiving 130 mg ABT-263 and 2 patients (1 NSCLC and 1 carcinoid) receiving 325 mg ABT-263. Two drug-related DLTs (grade 4 thrombocytopenia) were observed at 325 and 425 mg. Another DLT of fatal respiratory failure occurred at 10 mg (30).

The activities of ABT-263 in patients with relapsed or refractory lymphoid malignancies (lymphoma and follicular lymphoma) will be evaluated in another nonrandomized, open-label, uncontrolled phase I/IIa clinical trial sponsored by Genentech. The purpose of the phase I portion of the trial is to evaluate the pharmacokinetic and safety profiles of ABT-263 with the objective of defining the DLT and MTD, and the purpose of the phase IIa portion of the trial is to further evaluate the safety and preliminary efficacy of ABT-263 at the dose recommended from the phase I portion of the study. The trial is currently recruiting participants (26).

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Abbott Laboratories; developed in collaboration with Genentech.

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