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OLAPARIB

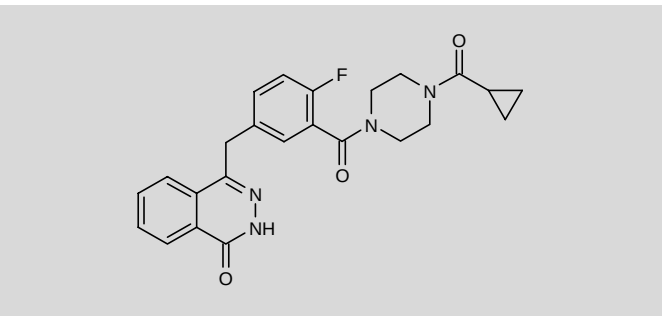
Rec INN

PARP-1 and PARP-2 Inhibitor
Oncolytic

AZD-2281
KU-0059436
KU-59436

4-[3-[4-(Cyclopropylcarbonyl)piperazin-1-ylcarbonyl]-4-fluorobenzyl]phthalazin-1(*2H*)-one

InChI=1/C24H23FN4O3/c25-20-8-5-15(14-21-17-3-1-2-4-18(17)22(30)27-26-21)13-19(20)24(32)29-11-9-28(10-12-29)23(31)16-6-7-16/h1-5,8,13,16H,6-7,9-12,14H2,(H,27,30)



$C_{24}H_{23}FN_4O_3$
Mol wt: 434.4628
CAS: 763113-22-0
EN: 417695

ABSTRACT

Olaparib is a potent inhibitor of poly [ADP-ribose] polymerases PARP-1 and PARP-2 that is currently undergoing clinical development for the treatment of BRCA1/2-defective breast, ovarian, pancreatic and colorectal tumors and melanoma. Olaparib has the potential for use as a single agent or in combination with platinum-based DNA-damaging agents and cytotoxic drugs, as well as radiotherapy. Preliminary clinical data support the safety and oral bioavailability of the compound and warrant its further development as a potential clinical candidate in cancer therapy.

SYNTHESIS

Olaparib can be prepared as follows. Reaction of 2-carboxybenzaldehye (I) with dimethyl phosphite in the presence of MeONa in MeOH affords the isobenzofuranylphosphonate (II), which is condensed with 2-fluoro-5-formylbenzonitrile (III) by means of triethylamine in THF to give the benzylidene-isobenzofuranone (IV). Basic hydrolysis of the nitrile group of compound (IV) followed by treatment with hydrazine hydrate leads to the phthalazinone-carboxylic acid (V) (1-3), which can also be obtained by treatment of isobenzofuranone (IV) first with hydrazine hydrate in THF, followed by hydrolysis of the resulting phthalazinone nitrile (VI) with aqueous NaOH (*2*). Coupling of carboxylic acid (V) with *N*-Boc-piperazine (VII) by means of HBTU and DIEA in dimethylacetamide yields the Boc-protected amide (VIII), which is then deprotected to compound (IX) by treatment with HCl in EtOH or MeOH. Finally, piperazine-phthalazinone (IX) is acylated with cyclopropanecarbonyl chloride (X) in the presence of triethylamine or DIEA in CH_2Cl_2 (1, *2*). Alternatively, condensation of piperazine (XI) with cyclopropanecarbonyl chloride (X) in AcOH solution gives *N*-(cyclopropylcarbonyl)piperazine (XII) (*2*), which is then coupled with the phthalazinone-carboxylic acid (V) by means of HBTU and DIEA in acetonitrile or dimethylacetamide (*2*, 3). Scheme 1.

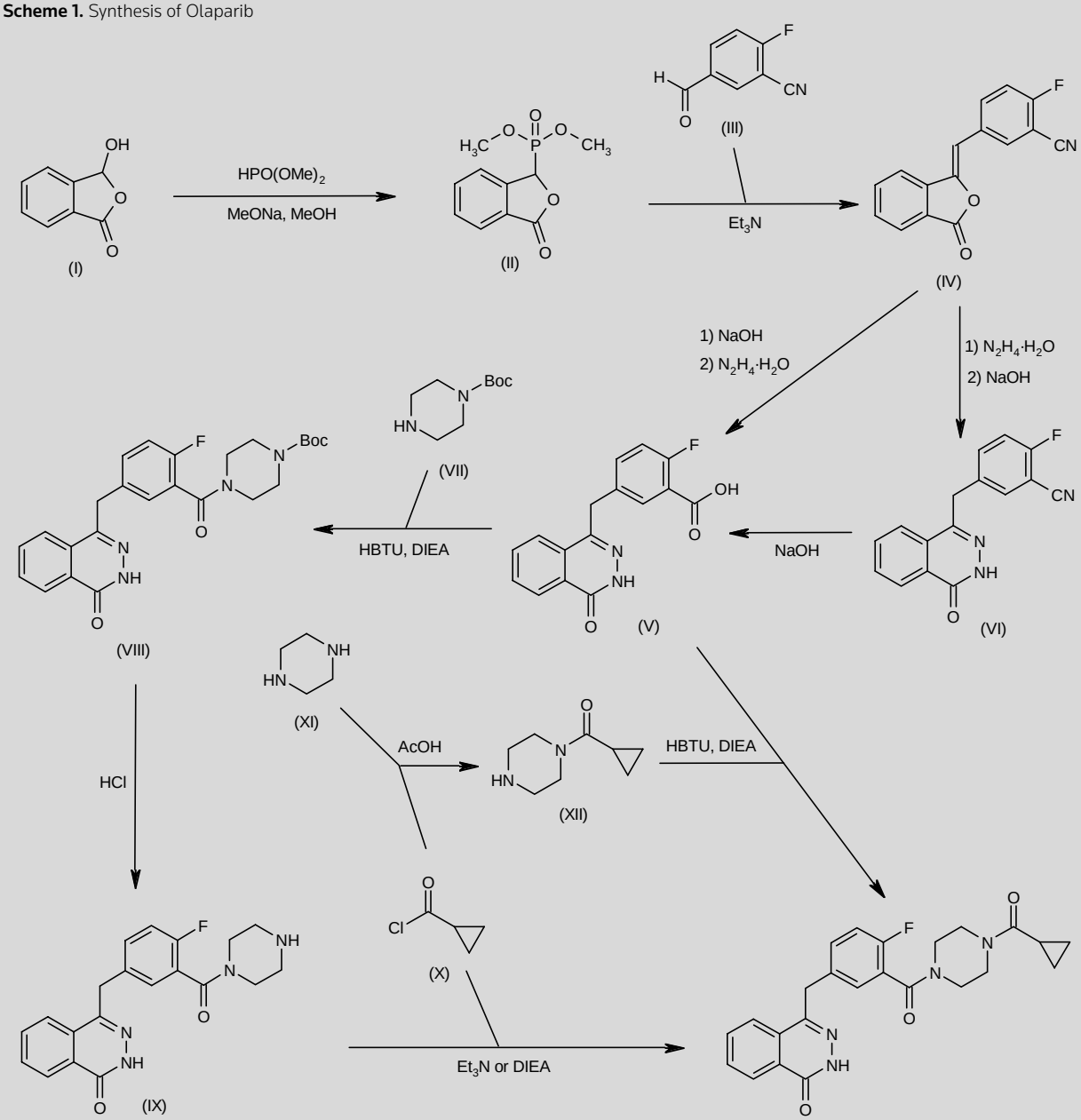
BACKGROUND

Poly [ADP-ribose] polymerases PARP-1 and PARP-*2* are abundant nuclear enzymes activated by DNA damage in the form of single-strand (ss) DNA breaks. PARP-1 and -*2* play a key role in the repair of ssDNA breaks via the base excision repair (BER) pathway (4). Several anticancer therapies operate by inducing breaks in the DNA chain, which, if not repaired, may lead to cell death. PARP inhibition may therefore represent a possible means to increase the efficiency of certain cytotoxic treatments (5).

It has recently been demonstrated that PARP inhibitors may be employed to selectively destroy cancer cells that are defective in a different DNA repair mechanism. Breast and ovarian cancer cells harboring mutations in the breast cancer-associated genes *BRCA1* and *BRCA2* are defective in the homologous recombination (HR) DNA repair pathway of double-strand (ds) damaged DNA. Failure to repair ssDNA breaks due to inhibition of PARP leads to persistence of these lesions and their subsequent conversion to dsDNA breaks,

S. Vasiliou, R. Castañer, J. Bolós.
Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Olaparib



following DNA replication. Normal cells are able to redeem this situation by employing the HR repair pathway, whereas *BRCA1/2*-deficient cells are selectively destroyed due to a nonfunctional HR repair mechanism (6). PARP inhibition may confer selective cytotoxicity to tumor cells with attenuated *BRCA1/2* function, without affecting normal cells. This selective exploitation of DNA repair mechanisms is considered a major breakthrough in cancer treatment and renders PARP-1 and -2 major targets in cancer therapy.

Inhibitors of PARP have been shown to enhance the cytotoxic effects of ionizing radiation and chemotherapeutic drugs used in cancer treatment regimens (7) and may also be used as single agents to promote the death of cancer cells defective in *BRCA*-mediated DNA repair (8). The development of selective PARP-1 and -2 inhibitors with high efficacy and low toxicity has been an ongoing effort. Currently, AstraZeneca is conducting clinical trials with olaparib (AZD-2281, KU-59436, KU-0059436), an oral inhibitor of PARP originally developed by its wholly owned subsidiary KuDOS Pharmaceuticals. The safety and tolerability of olaparib are also being assessed in combination with platinum-based DNA-damaging agents such as cisplatin and carboplatin, as well as cytotoxic agents including gem-

citabine, topotecan, dacarbazine, irinotecan and paclitaxel, in clinical trials for breast, ovarian, pancreatic and colorectal tumors and melanoma.

PRECLINICAL PHARMACOLOGY

A novel series of substituted 4-benzyl-*2H*-phthalazin-1-one compounds were assessed for their enzyme-inhibitory activity and cellular potency against both PARP-1 and PARP-2, leading to the identification of a number of highly potent PARP inhibitors (3). The optimized compound, olaparib, was found to be essentially equipotent against PARP-1 (IC_{50} = 0.005 μM) and PARP-2 (IC_{50} = 0.001 μM) and was almost 300 times less effective against tankyrase (IC_{50} = 1.5 μM). The cellular potency of olaparib was confirmed in cultured human colon carcinoma SW620 cells, where it was found to potentiate methyl methanesulfonate-induced cell killing at concentrations ranging from 1 to 300 nM (potentiation plateau observed at 100 nM). Compound inhibited PARP-1 in SW620 cell lysates with an IC_{50} of 6 nM and total ablation of PARP-1 activity was observed at concentrations of 30-100 nM. Olaparib also displayed the ability to potentiate the antitumor activity of the methylating chemotherapeutic agent temozolomide in a mouse model of SW620 xenografts. Olaparib (10 mg/kg p.o.) administered in combination with temozolomide (50 mg/kg p.o.) once daily for 5 consecutive days induced a significant reduction (> 80%) in tumor volumes throughout the study compared to treatment with temozolomide alone. Combination therapy was well tolerated under the dosing regimen employed, with no exacerbation of the systemic toxicity of temozolomide and no mortality.

The efficacy of olaparib was assessed in vitro against a panel of newly established *BRCA2*-deficient mouse mammary tumor cell lines derived from the KB2P mouse strain and compared to *BRCA2*-proficient tumors (mouse strain KP) (9). Olaparib was found to strongly inhibit the growth of *BRCA2*-deficient cell lines (average IC_{50} = 91 nmol/L) compared to *BRCA2*-proficient cell lines (average IC_{50} = 8.135 nmol/L). It was also shown to cause the strongest differential inhibition of *BRCA2*-deficient cell lines compared to 11 different anticancer drugs (including cytotoxic agents that directly induce DNA strand lesions such as mitomycin C, methyl methanesulfonate, temozolomide and cisplatin) and gamma radiation. The possibility of synergy or additive activity between the platinum-based agent cisplatin and the PARP inhibitor olaparib was also addressed in the same in vitro model. Both agents are able to generate dsDNA breaks and their combination might enhance the efficacy of monotherapy and reduce the toxic side effects caused by platinum-based drugs. Synergistic cytotoxicity between olaparib and cisplatin was demonstrated in *BRCA2*-deficient but not *BRCA2*-proficient mammary tumor cell lines. The study supports further preclinical evaluation of olaparib as monotherapy or in combination with cisplatin in animal models of *BRCA2*-deficient breast cancer.

The radiosensitizing effects of PARP inhibition were also investigated in vitro in an attempt to evaluate the therapeutic potential of olaparib in human glioblastoma multiforme, the most common and aggressive primary brain tumor, with a very low survival rate (10). Four human glioblastoma cell lines of diverse genetic backgrounds (T98G, U-373 MG, UVM and U-87 MG) were employed. Exposure of these cell lines to noncytotoxic concentrations of olaparib (up to 5 μM) that were able to inhibit PARP activity resulted in a marked reduction of cell survival following single doses of gamma radiation in all cell lines except U-87 MG. The sensitizer enhancement ratios at 37% (SER37) and 50% (SER50) survival ranged between 1.38 and 1.68 and were found to correlate with DNA replication (calculated as percentage of cells in S phase). T98G cells in S phase exposed to the DNA polymerase inhibitor aphidicolin (2 μM) during a 3-h treatment with olaparib (1 μM) displayed a reduced sensitizing effect (SER50 = 1.09 compared to SER50 = 1.34 in the absence of aphidicolin). A combination of olaparib with a fractionated, clinically relevant radiation schedule resulted in enhancement of radiosensitization in T98G cells. Fractionation of radiation (four doses of 2 Gy) reduced the cytotoxicity observed following administration of single higher doses and resulted in an increase in the radiosensitizing effects of olaparib (1 μM) compared to its effects measured with single-dose radiation treatments (SER37 = 1.56 and 1.26, respectively; SER50 = 1.55 and 1.27, respectively). The study concluded that concomitant administration of olaparib is likely to enhance the therapeutic potential of radiotherapy in glioblastoma multiforme treatment.

The in vitro cytotoxicity of PARP inhibition induced by olaparib was also addressed in 20 chronic lymphocytic leukemia (CLL) cells (11). CLL is a B-cell malignancy associated with an increase in the incidence of secondary neoplasms, including brain tumors and melanomas. The samples were subdivided into 10 carrying a mutant *ATM* (ataxia telangiectasia) gene and 10 wild-type *ATM* cells. The ATM protein is a major coordinator of the cellular response to dsDNA breaks; it controls the balance between dsDNA repair and the induction of the dsDNA apoptotic pathway. Mutant *ATM* CLL cells are deficient in both aspects of ATM function, i.e., repair and apoptosis. Noncycling and cycling states of CLL tumor cells were analyzed in order to ascertain potentially preferential toxic effects of PARP inhibition in the two CLL cell populations. An increased sensitivity to olaparib (1.5-10 μM) was observed in cycling CLL cells compared to noncycling populations. This elevated sensitivity in cycling CLL cells was evident in mutant *ATM* compared to wild-type *ATM* cells. The induction of ATM/p53-dependent apoptosis was not required in the killing of mutant *ATM* cells and neither caspase cleavage nor levels of p53 were affected by olaparib treatment. In noncycling CLL cells preincubation with olaparib for 24 h resulted in increased sensitivity of mutant *ATM* but not wild-type cells to subsequent administration of the DNA-damaging agent fludarabine. Preliminary data from this study support the potential use of olaparib in the treatment of CLL patients with apoptosis-resistant mutant *ATM* tumors.

The efficacy of olaparib was evaluated in vivo in a genetically engineered mouse model of *BRCA1*-associated breast cancer (12). Tumor growth inhibition (to approximately 40% of the initial size) was observed in tumor-bearing mice following treatment with olaparib (50 mg/kg i.p. for 28 days), without evident signs of toxicity. At this concentration olaparib was found to be rapidly absorbed and cleared. However, the intratumoral levels of olaparib compared with plasma concentrations at 2 and 6 h postinjection were estimated to be approximately 2- and 6- to 8-fold higher, respectively, which would indicate tumor loading of the drug. Prolonged treatment (100-day schedule) with olaparib significantly increased the median survival rate of mice from 60 to 131 days, without signs of toxicity. Although long-term administration of olaparib was preceded by the development of drug resistance, this effect could be reversed by coadministration of the P-glycoprotein inhibitor tariquidar (10 μL/g i.p.).

Olaparib was also found to potentiate the effects of the DNA-damaging platinum drug cisplatin. Coadministration of olaparib (50 mg/kg i.p. for 28- and 100-day cycles) with cisplatin (6 mg/kg i.v.) increased both the recurrence-free and overall survival of tumor-bearing mice compared to cisplatin monotherapy, although the combination also resulted in increased toxicity (12).

PHARMACOKINETICS AND METABOLISM

Olaparib displayed high absorption, measured as plasma concentrations at 30 and 60 min (0.23 and 0.59 µg/mL, respectively), following oral administration at 10 mg/kg in an initial medium-throughput in vivo pharmacokinetic assay performed in mice. The pharmacokinetic profile of olaparib and its oral bioavailability were further evaluated in rats and dogs. The compound was administered to rats at 15 mg/kg i.v. and p.o. and to dogs at 2.5 mg/kg i.v. and 10 mg/kg p.o. The half-life ($t_{1/2}$) was estimated to be 53 and 170 min, respectively, in rats and dogs. The oral bioavailability was 100% in both species, with dogs displaying lower relative clearance (Cl) 5.4 mL/min/kg compared to rats (49 mL/min/kg) (3).

CLINICAL STUDIES

The efficacy of olaparib in advanced solid tumors was evaluated in a phase I clinical trial carried out by KuDOS Pharmaceuticals in collaboration with research institutes in the U.K. and The Netherlands (13). Olaparib was administered to 12 patients with advanced solid tumors refractory to conventional treatment. Dose levels evaluated in the study were 10, 20, 40 and 80 mg/day and were associated with minimal toxicity (intermittent grade 1 nausea). Pharmacokinetic data obtained in this trial for doses up to 40 mg/day indicated dose proportionality, with a mean elimination $t_{1/2}$ of 6.91 h (range = 5.39.5 h), a mean clearance of 4.1 L/h (range = 1.3-9.4 L/h) and a mean volume of distribution of 39.8 L (range = 16.3-86.9 L). PARP inhibition of > 50% at 40 mg/day was observed in initial studies of tumor biopsies carried out pretreatment and on day 8 of olaparib treatment. Dose escalation in this trial is still ongoing.

Olaparib was also evaluated in cancer patients including *BRCA1/2* mutation carriers in a phase I clinical trial by KuDOS Pharmaceuticals and collaborators (14). A total of 44 patients (including 11 patients with *BRCA1/2* mutations and 2 patients with strong family histories suggesting *BRCA1/2* mutations) participated in the trial. Dose levels tested were 10, 20, 40 and 80 mg administered once daily. Subsequently, 60- and 100-mg doses were administered twice daily for 2 of 3 weeks, followed by 100, 200, 400 and 600 mg twice a day continuously. Toxicities associated with olaparib administration included grade 1-2 fatigue, anorexia, constipation and diarrhea. Dose proportionality was supported by pharmacokinetic data up to 200 mg twice daily. The mean elimination $t_{1/2}$ was estimated at 6 h (range = 4.6-7 h), mean clearance/bioavailability (F) was 4.55 L/h (range = 1.8-9.1 L/h) and mean volume of distribution/F was 39.9 L (range = 17-92.1 L). A significant inhibition of PARP functional activity (> 50%) was observed in peripheral blood mononuclear cells and tumor tissues at doses of > 40 mg administered once a day. A final dose level of 600 mg twice daily is currently being evaluated in an effort to maximize PARP inhibition in tumor cells.

Preliminary data from a phase I clinical trial of olaparib performed at the Royal Marsden Hospital in the U.K. indicated good tolerability and demonstrated significant activity in patients with *BRCA*-associated ovarian cancer (15).

A number of phase I and I/II clinical trials evaluating the efficacy, safety and tolerability of olaparib as a single agent or in combination with chemotherapeutic drugs in anticancer treatments are currently ongoing (16-33).

SOURCE

AstraZeneca (GB).

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