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MONOGRAPH

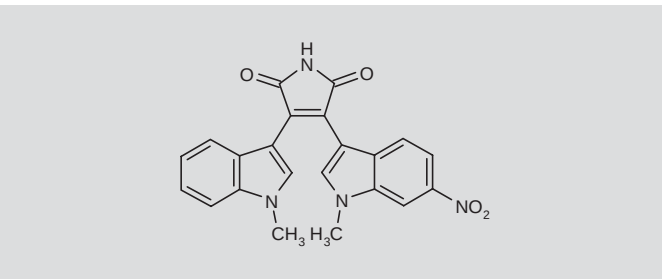
MKC-1

Antimitotic Agent
Apoptosis Inducer
Oncolytic

Ro-31-7453 (former code name)

3-(1-Methyl-1*H*-indol-3-yl)-4-(1-methyl-6-nitro-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione

InChI=1/C22H16N4O4/c1-24-10-15(13-5-3-4-6-17(13)24)19-20(22(28)23-21(19)27)16-11-25(2)18-9-12(26(29)30)7-8-14(16)18/h3-11H,1-2H3,(H,23,27,28)



$C_{22}H_{16}N_4O_4$
Mol wt: 400.3868
CAS: 125313-92-0
EN: 287215

ABSTRACT

MKC-1 (formerly known as Ro-31-7453) is a novel, orally active cell cycle inhibitor with broad-spectrum antitumor effects, including multidrug-resistant cell lines. Preclinical investigations have shown that MKC-1 produces antiproliferative activity via G2/M arrest, apoptosis and antiangiogenesis. Successful clinical outcomes have been reported in phase I and II trials in refractory and advanced cancer patients, along with combination therapy studies. EntreMed and the National Cancer Institute are currently undertaking further phase I/II trials with MKC-1.

SYNTHESIS

Methylation of 6-nitroindole (I) using either iodomethane and NaH (1, 2) or dimethyl carbonate and Na_2CO_3 (3) provides 1-methyl-6-nitroindole (II), which is subsequently acylated with oxalyl chloride in CH_2Cl_2 to furnish the glyoxylyl chloride (III). Condensation of chloride (III) with 1-methylindole-3-acetic acid (IV) in the presence of Et_3N gives the bis(indolyl)maleic anhydride (V), which is then converted to the target maleimide derivative by heating with ammonia in aqueous DMF in a sealed vessel at 140 °C (1, 2). Scheme 1.

In a related procedure, quaternization of 3-(dimethylaminomethyl)-indole (VI) with butyl bromide, followed by amine displacement with NaCN yields 2-(3-indolyl)acetonitrile (VII), which is alkylated using dimethyl carbonate and K_2CO_3 in hot DMF to give the 1-methylindole derivative (VIII). Treatment of compound (VIII) with isopropanol and HCl (generated from acetyl chloride and *i*-PrOH) affords imidate (IX), which is finally condensed with the indoleglyoxylyl chloride (III) in the presence of DIEA, followed by treatment with H_2SO_4 (3). Scheme 1.

BACKGROUND

Cancer is a growing concern worldwide (4, 5). The World Health Organization (WHO) has indicated that cancer causes approximately 13% of all deaths (6). Therapies targeted to deregulated proteins specific to cancer cells have been emerging since the late 1990s and represent a promising treatment avenue for multiple types of cancer. One of the main causes of failure in the treatment of cancer is the development of drug resistance (7). This is a very serious problem that may lead to recurrence of disease or even death. Oncolytic therapeutic strategies are now favoring pharmaceuticals that are multi-targeting, i.e., target multiple proteins or signaling pathways that are associated with abnormal functioning in tumor cells, as it is almost universally the case that single-target drugs will encounter resistance.

MKC-1 (formerly known as Ro-31-7453) is a novel, orally active cell cycle inhibitor with broad-spectrum antitumor effects. This agent is currently undergoing clinical development, with four U.S. phase I and II trials recently completed by EntreMed in advanced and metastatic breast cancer, refractory hematological malignancies and unresectable or metastatic pancreatic cancer (8-10) and by the National Cancer Institute (NCI) at the Memorial Sloan-Kettering Cancer Center in colorectal cancer (11). Active (but not recruiting) trials include two studies sponsored by EntreMed, i.e., a phase II study in patients with ovarian or endometrial cancer in Canada (12) and a phase I/II trial of MKC-1 combined with pemetrexed to treat advanced cancer and non-

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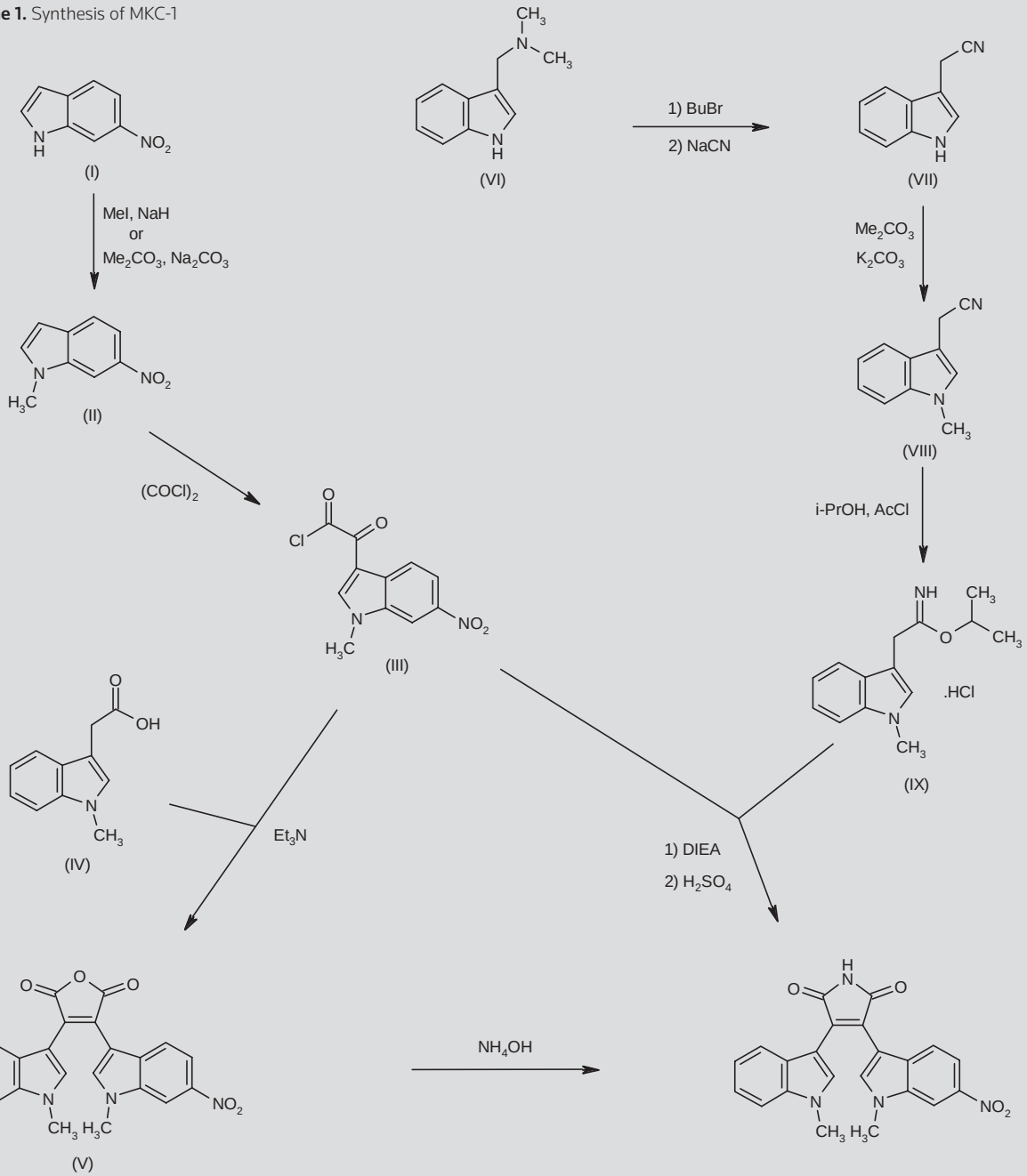
Scheme 1. Synthesis of MKC-1



small cell lung cancer (NSCLC) in the U.S. (13), as well as two phase I studies at the Memorial Sloan-Kettering Cancer Center and the NCI in metastatic solid tumors (14, 15). EntreMed is currently actively recruiting for a phase I trial in patients with advanced cancer (16).

PRECLINICAL PHARMACOLOGY

Studies have evaluated the activity of MKC-1 against a panel of kinases. It was shown that GSK-3β was the only kinase significantly inhibited by MKC-1 (IC_{50} = 7 nM). Nonenzymatic targets for MKC-1 have also been identified, with associated aberrant mitotic spindle formation and cell cycle arrest along with binding to importin-beta and colchine binding sites of tubulin. Moreover, MKC-1 has been found to induce a reduction in the activity of the transcription factor HIF-1α and activation of the transcriptional activity of phosphorylated nuclear factor NF-kappa-B and STAT3 after 24-h treatment in human cell lines (17).

In vitro, at antiproliferative concentrations (< 1 μM) MKC-1 induced apoptosis by preventing normal progression through the M phase by inhibiting the formation of the mitotic spindle. At higher concentrations (10 μM), the antiapoptotic activity of MKC-1 was associated with inhibition of progression into the S phase (18, 19).

Studies in the human renal cell carcinoma cell line Caki-1 have shown that MKC-1 treatment results in inhibition of phosphorylated mTOR and its downstream target p70-S6K. Further evidence indicated that MKC-1 treatment of Caki-1 cells provides a concentration- and time-dependent reduction in phosphorylated Akt, but not phosphorylated PDK1, without inhibiting phosphatidylinositol 3-kinase (PI3K), PDK1 or Akt itself, suggesting that MKC-1 inhibits the Akt/mTOR pathway (20).

In vitro efficacy against at least 30 human tumor cell lines has been demonstrated (IC_{50} = 0.02-0.30 μM), including all 5 multidrug-resistant cell lines tested (21). This antiproliferative activity has been shown to be independent of tissue type, p53 function or estrogen receptor status (18).

The antiproliferative activity of MKC-1 has been assessed against a panel of hematopoietic cell lines. MKC-1 showed potent and concentration-dependent activity against HL-60, U-937, MV-4-11, THP-1, Jurkat and OCI-AML 1-5 cell lines, with IC_{50} values in the range of 20-400 nM. MKC-1 also inhibited the growth of primary cells derived from acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) patients. Additionally, MKC-1 showed enhanced activity when combined with the chemotherapeutic agent cytarabine (Ara-C) when added simultaneously or sequentially using the OCI-AML 4 cell line (22).

MKC-1 has also demonstrated synergistic antitumor activity with paclitaxel in cell culture studies. This was most obvious in the human breast carcinoma MDA-MB-435 cell line, where it provided 10-15% growth inhibition while combined administration gave > 80% inhibition (23).

Two metabolites of MKC-1 (Ro-27-0431 and Ro-27-4006) exhibited similar potency to MKC-1 in inhibiting the growth of MDA-MB-435 cells and five multidrug-resistant human tumor cell lines (24).

MKC-1 has been shown to cause significant growth inhibition at well-tolerated doses in several breast, colorectal, NSCLC and prostate cancer xenografts in nude mice, including drug-resistant models. Administration i.p., p.o. or by continuous infusion at nontoxic doses provided significant tumor growth inhibition or regression. MKC-1 also produced significant inhibition of subcutaneous rat mammary adenocarcinoma MTLn3 tumors and lung lesions and caused regression in the transgenic *Min* mouse model, with additional prevention of the formation of intestinal polyps (25).

More detailed results on the effect of MKC-1 on the primary and metastatic growth of MTLn3 rat mammary adenocarcinoma were reported. MKC-1 markedly suppressed the growth of primary tumors, with a %T/C of 34.3 and 12.2 when given at doses of 12.5 and 25 mg/kg i.p. b.i.d., respectively, 5 times a week over a period of 3 weeks. MKC-1 also reduced the overall incidence of spontaneous regional, axillary, inguinal and para-aortic lymph node metastases by 42% and 59%, respectively. The higher dose also significantly reduced the formation of experimental lung metastases (–59%) (26).

In vivo studies have shown positive results for i.v. infusion of MKC-1. In nude mice bearing human MDA-MB-435 breast cancer xenografts, 80-100% tumor regression was demonstrated with 4-day infusions at doses of 50-100 mg/kg/day, with 40-100% regression also noted after 7-day infusions at the same dose range. The highest dose was, however, lethal in 60% of the animals after 7-day infusion. In additional studies in RKO colorectal xenografts, MKC-1 (50-100 mg/kg/day) produced 60% tumor regressions after 3 and 10 days of infusion. In these studies continuous i.v. infusion was more effective than daily i.p. administration (27).

Studies in mice bearing human renal cell carcinoma Caki-1 xenografts have shown that daily oral treatment with MKC-1 (200 mg/kg) significantly increased the median survival time (MST) of the animals. The MST for the control animals was 49.5 days, with no animals surviving on day 88. In contrast, MKC-1-treated animals showed an MST of 88 days (20).

Using two human xenograft models in nude mice, researchers investigated whether repeated MKC-1 treatments are associated with resistance or sensitivity to toxicity. In HT-29 and MDA-MB-435 xenografts it has been shown that tumors were at least as sensitive to MKC-1 following administration of a second course of treatment, without any significant additional toxicity (28).

The antiangiogenic activity of MKC-1 was investigated in the corneal pocket model in mice. Oral administration of MKC-1 at 100 and 200 mg/kg was shown to inhibit basic fibroblast growth factor (bFGF)- and vascular endothelial growth factor (VEGF)-induced mouse corneal angiogenesis by 49-66% and 65-75%, respectively. However, toxicity also accompanied oral dosing. Antiangiogenic activity was also seen for i.p. dosing, without any associated toxicity (29).

As seen in in vitro studies, enhanced efficacy has been shown when combining MKC-1 and paclitaxel in vivo. I.p. administration of MKC-1 and paclitaxel significantly slowed tumor growth in athymic mice implanted with MDA-MB-435 cells, with little evidence of toxicity (23).

Studies in SCID mice with established MDA-MB-231 human breast carcinoma xenografts have shown that after 14 days of treatment tumor growth inhibition with either MKC-1 (200 mg/kg/day p.o.) or paclitaxel alone (20 mg/kg i.p. on study days 1, 3 and 21) was recorded as 38% and 76%, respectively. In contrast, tumor regression occurred in mice treated with the combination of the two agents, with an interaction index indicating that the combination was synergistic. After 31 days of treatment, tumor volumes of 2000 mm^3 were reported for single treatment regimens, whereas mean tumor volume in mice receiving combined treatment was 400 mm^3. Analysis of ex vivo tumor biopsies has also shown that antiproliferative (Ki67), antiangiogenic (CD31) and proapoptotic (TUNEL and cleaved caspase-3) activity is increased in tumors treated with MKC-1 and paclitaxel compared to either agent alone. Similarly, the oncogenic proteins HIF-1α, phosphorylated Akt, phosphorylated S6 kinase and phosphorylated STAT-3 were further decreased in tumors treated with MKC-1 in combination with paclitaxel (30).

The antiproliferative activity of MKC-1 was investigated in combination with the nucleoside analogue gemcitabine in mice bearing human NSCLC A549 xenografts. The antitumor activity of the opti-

mal combination (simultaneous p.o. administration of 100 mg/kg MKC-1 given every 12 h for 21 days plus i.p. administration of 60 mg/kg of gemcitabine every 3 days for 5 doses) was better than single-agent gemcitabine at its MTD (%T/C = 18 vs. 61) (31).

Combination studies with capecitabine plus MKC-1 (both given p.o.) have been carried out in MDA-MB-435 and colon carcinoma HCT 116 xenograft models in mice. In the breast xenograft model tumor growth inhibition was significantly greater for combined versus single-agent administration (%T/C combined = 17; capecitabine = 91; MKC-1 = 28), without toxicity. In the colon xenograft model, combination therapy also yielded more potent antitumor activity (%T/C combined = 1; capecitabine = 14; MKC-1 = 20) (32).

Dose-escalation studies in tumor-bearing nude mice have also shown that the efficacy of oral doses (25-100 mg/kg b.i.d. over 9-15 days) of MKC-1 is dose-dependent. Furthermore, maximum antitumor efficacy and minimum toxicity are seen when the total doses over a treatment period are kept constant, with the lowest daily dose administered for the longest treatment period (33). Computational simulation studies in mice administered MKC-1 p.o. and i.p. indicated that antitumor activity may be enhanced by increasing time of effective drug exposure (34).

The effects of one of the major metabolites of MKC-1 have also been investigated in vivo. Ro-27-0431 was shown to inhibit the growth of rat mammary adenocarcinomas by 97.5% when administered as a continuous i.v. infusion of 72 mg/kg/day over a period of 1 week. Continuous dosing was associated with only a minimal decrease in body weight (24).

PHARMACOKINETICS AND METABOLISM

Studies conducted in liver microsomes from mice, rats, dogs, monkeys and humans have shown that MKC-1 metabolites are similar across species and indicate that its major metabolic pathway is *N*-demethylation. The five major in vitro metabolites were identical to those found in animals in vivo. Intrinsic clearance from the microsome study, expressed as V_{max}/K_m (mL/min/mg microsomal protein), followed the order: monkey (1.26) > rat (0.73) > mouse (0.33) > human (0.17) > dog (0.10), suggesting that the differences in bioavailability were attributed mainly to species differences in the degree of hepatic first-pass metabolism. Marked species-dependent pharmacokinetic properties were also observed for MKC-1 (single doses of 1 mg/kg i.v.). Plasma clearance was much greater in mice and rats than in dogs and monkeys, and the corresponding plasma elimination half-life was approximately 1 h in mice and rats but > 5 h in dogs and monkeys; there was less interspecies variation in volume of distribution and plasma protein binding. Metabolite profiles were similar for all species. The oral bioavailability of the drug was 12%, 19%, 25% and 84%, respectively, in monkeys, mice, rats and dogs following a single oral dose of 10 mg/kg (35).

Results from phase I clinical trials in patients with refractory solid tumors treated with MKC-1 at oral doses of 25-800 mg/m^2 once daily or 400 mg/m^2 b.i.d. for 4, 7 or 14 days indicated that the drug was rapidly absorbed (t_{max} = 4 h), with a half-life of about 9 h, supporting a twice-daily dosing regimen. The parent compound and its two major metabolites (Ro-27-4006 and Ro-27-0431) showed linear exposure (36).

CLINICAL STUDIES

A dose-finding phase I study of oral MKC-1 given every 12 h for either 7 or 14 consecutive days repeated every 4 weeks was carried out in 37 patients with refractory cancer (n = 14 on the 7-day schedule, n = 23 on the 14-day schedule). After a median of 6 cycles on 100, 200, 240 and 280 mg/m^2 b.i.d. for 7 days and 70, 100, 125 and 150 mg/m^2 b.i.d. for 14 days, myelosuppression and mucositis were reported as dose-limiting. The maximum tolerated doses were 200 and 125 mg/m^2 b.i.d., respectively, for the 7- and 14-day schedules. Pharmacokinetic analysis showed rapid absorption and metabolism. The area under the concentration–time curve (AUC) and trough concentrations of MKC-1 and the two active metabolites appeared to be dose-proportional, with a half-life of approximately 9 h and a t_{max} of approximately 4 h. One patient with pretreated lung cancer had a partial response (37-39).

A phase I accelerated dose-escalation study was performed in 43 patients with advanced solid tumors treated with doses of 25-800 mg/m^2/day once or twice daily for 4 consecutive days every 3 weeks. Myelosuppression and stomatitis were reported as dose-limiting toxicities in 5 of 14 patients on the highest dose. Other common adverse events included diarrhea, nausea, vomiting, fatigue and alopecia. The pharmacokinetics of the parent compound and major metabolites were linear, with a half-life of approximately 9 h and a t_{max} of approximately 4 h. Possible antitumor activity was observed in patients with cancer of the lung, breast, pancreas and ovary (40, 41).

Data from an open-label phase I/II study of MKC-1 in combination with pemetrexed (Alimta®) in advanced NSCLC patients were used to define a recommended combination dose and the antitumor activity (progression-free survival) of this combination. In eight patients involved in the phase I dose-finding study, MKC-1 (75 mg/m^2) and pemetrexed (500 mg/m^2) were not associated with dose-limiting toxicities; all-grade toxicities included fatigue (75%), anorexia (63%), nausea, vomiting and dyspnea (all 50%), and neutropenia (25%). Of the 11 patients involved in the phase II tumor response study, 7 were evaluable for tumor response. The best response at the time of reporting was stable disease, which was seen in all seven patients. Interestingly, one patient (whose disease progressed after carboplatin/paclitaxel/bevacizumab and erlotinib treatments) showed stable disease for over 8 months and remained on MKC-1 monotherapy at the time of publication with minimal toxicity (mild fatigue) (42).

A phase I study of MKC-1 given orally every 12 h on days 1-4 and paclitaxel given i.v. over 3 h on day 1 every 3 weeks in patients with advanced solid tumors (N = 30; NSCLC, n = 15; head and neck cancer, n = 9; mesothelioma, n = 5; thymoma, n = 1) has shown an acceptable safety profile, with grade 3-4 events reported as neutropenia, fatigue, nausea, emesis, mucositis, dyspnea, constipation and arthralgia; no treatment-related deaths were recorded. The maximum tolerated doses of MKC-1 and paclitaxel were 220 and 150 mg/m^2, respectively. Pharmacokinetic analyses suggested no MKC-1/paclitaxel interaction. After a median of 5 treatment cycles 1 complete response and 12 cases of stable disease were reported (43, 44).

Preliminary phase I data have also been published for MKC-1 administered in combination with gemcitabine in patients with various primary cancers. Stable disease was evident in 8 patients after 24

weeks of therapy with MKC-1 at 220 mg/m^2 given every 12 h for 4 days and gemcitabine 1000 mg/m^2 on days 1 and 8 every 3 weeks. Grade 3-4 adverse events (thrombocytopenia, neutropenia, hepatotoxicity, anorexia, mucositis and dyspnea) were reported in nine patients, with no evidence of treatment-related deaths. High pharmacokinetic variability was observed and terminal half-lives of MKC-1 and its two active metabolites (Ro-27-4006 and Ro-27-0431) averaged 4-8 h on the last day of treatment (45).

The efficacy and safety of MKC-1 were later examined in a phase II study in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. Data from 58 patients demonstrated that MKC-1 was well tolerated: grade 3 or greater drug-related toxicities included elevated AST/ALT (7%), sensory neuropathy (2%), elevated creatinine (2%), mucositis (2%) and neutropenia (7%), including 1 death from neutropenic sepsis. Several patients discontinued due to toxicity. Of those patients proceeding to cycle 2, 51% and 11%, respectively, had the dose increased or reduced. Of the 49 patients evaluable for response, the overall response rate was 6.1% and an additional 14% had stable disease (46, 47).

SOURCE

EntreMed, Inc. (US).

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