Drugs of the Future 2009, 34(8): 624-633

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.8.1400202

ZIBOTENTAN

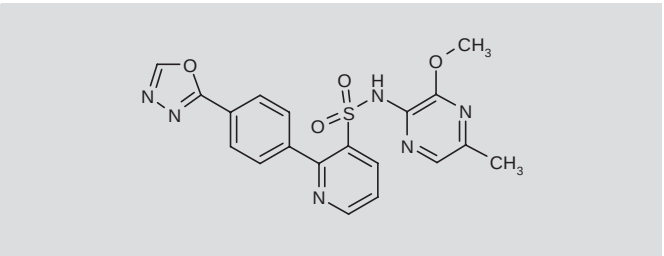
Rec INN; USAN

Endothelin ET_A Receptor Antagonist
Oncolytic

ZD4054

N-(3-Methoxy-5-methylpyrazin-2-yl)-2-[4-(1,3,4-oxadiazol-2-yl)phenyl]pyridine-3-sulfonamide

InChI=1S/C19H16N6O4S/c1-12-10-21-17(19(23-12)28-2)25-30(26,27)15-4-3-9-20-16(15)13-5-7-14(8-6-13)18-24-22-11-29-18/h3-11H,1-2H3,(H,21,25)



$C_{19}H_{16}N_6O_4S$
Mol wt: 424.433
CAS: 186497-07-4
EN: 258506

ABSTRACT

Endothelin-1 (ET-1) is a 21-amino-acid peptide factor that binds two receptors, ET_A and ET_B, which exert distinct biological effects. Zibotentan (ZD4054) is a specific ET_A receptor antagonist in clinical development for the treatment of hormone-resistant prostate cancer (HRPC). In preclinical studies, zibotentan inhibited ET-1-mediated changes in cellular invasiveness in vitro, and inhibited angiogenesis, metastasis and the growth of tumor xenografts in vivo. Consistent with its specific binding profile, zibotentan inhibited ET_A receptor-mediated antiapoptotic events, while allowing ET_B receptor-mediated proapoptotic signaling. In the clinical setting, the absence of zibotentan activity at the ET_B receptor was demonstrated by the stability of circulating ET-1 levels following single zibotentan doses up to 240 mg. In a randomized, placebo-controlled phase II trial in patients with pain-free or mildly symptomatic metastatic HRPC (N = 312), zibotentan was generally well tolerated, with an adverse effect profile consistent with its known pharmacological activity, the most common adverse events being headache, peripheral edema and nasal congestion. Although there was no significant difference between zibotentan treatment groups and placebo for the primary endpoint of time to progression, there was a promising signal for prolonged overall survival with zibotentan. These results support the strategy of targeting the ET_A receptor in prostate cancer. Phase III trials are under way.

SYNTHESIS**

Zibotentan can be prepared by two alternative routes.

In one strategy, condensation of 4-bromobenzohydrazide (I) with $HC(OEt)_3$ in the presence of H_2SO_4 in refluxing methylated spirit gives 2-(4-bromophenyl)-1,3,4-oxadiazole (II), which upon treatment with $(i\text{-PrO})_3B$ in the presence of MeLi and BuLi in THF at −65 °C affords the boronic acid derivative (III). Suzuki coupling between the boronic acid (III) and the chloropyridine derivative (IV) in the presence of $Pd(OAc)_2$, 3,3′,3″-phosphinidynetris(benzenesulfonic acid) trisodium salt (TPPTS) and NMM in H_2O/*i*-PrOH at 83 °C affords *N*-protected zibotentan (V). Finally, intermediate (V) is deprotected with ethanolamine in H_2O/*i*-PrOH at 42 °C or with NH_3 in H_2O/methylated spirit at 60 °C and acidified with AcOH in H_2O (1). Scheme 1.

In a second strategy, 4-carboxyphenylboronic acid (VI) is esterified with MeOH in the presence of H_2SO_4, yielding boronic acid (VII), which undergoes Suzuki coupling with the chloropyridine derivative (IV) in the presence of $Pd(PPh_3)_4$ and KF in refluxing toluene/H_2O to provide adduct (VIII). Finally, methyl ester (VIII) is converted to hydrazide (IX) by treatment with hydrazine hydrate in refluxing methanol and cyclized by condensation with refluxing $HC(OEt)_3$ (2). Scheme 1.

Intermediate (IV) can be prepared as follows.

Bromination of 2-amino-5-methylpyrazine (X) with Br_2 in $CHCl_3$ affords the bromopyrazine (XI), which by subsequent bromide displacement by means of sodium methoxide in refluxing methanol gives the methoxypyrazine (XII). Then, the amino group of (XII) is protected by acylation with isobutyl chloroformate (XIII) in the presence of pyridine in CH_2Cl_2 to provide carbamate (XIV), which finally, after being pretreated with NaH, is sulfonylated with 2-chloropyridine-3-sulfonyl chloride (XV) in DMF. Compound (XV) is prepared

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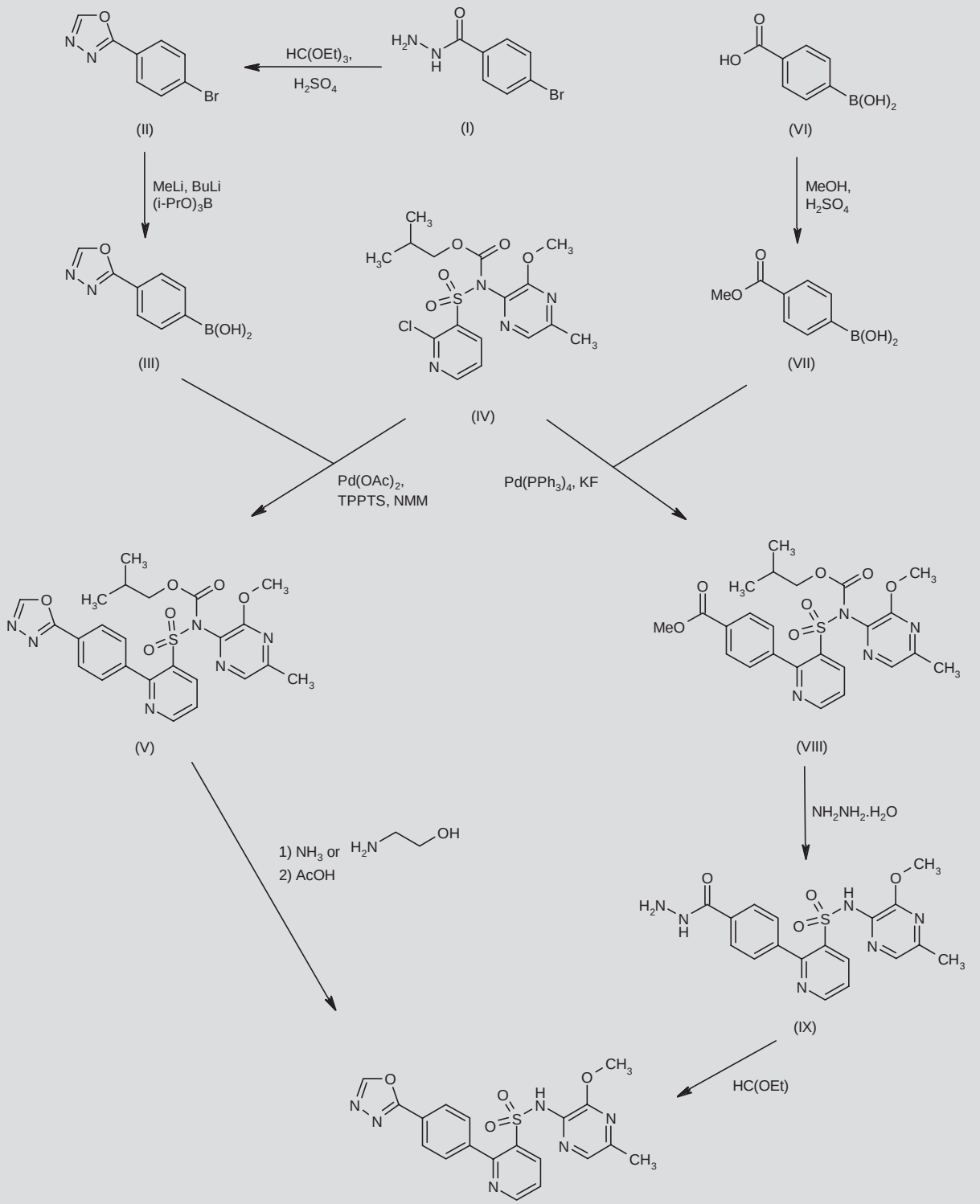
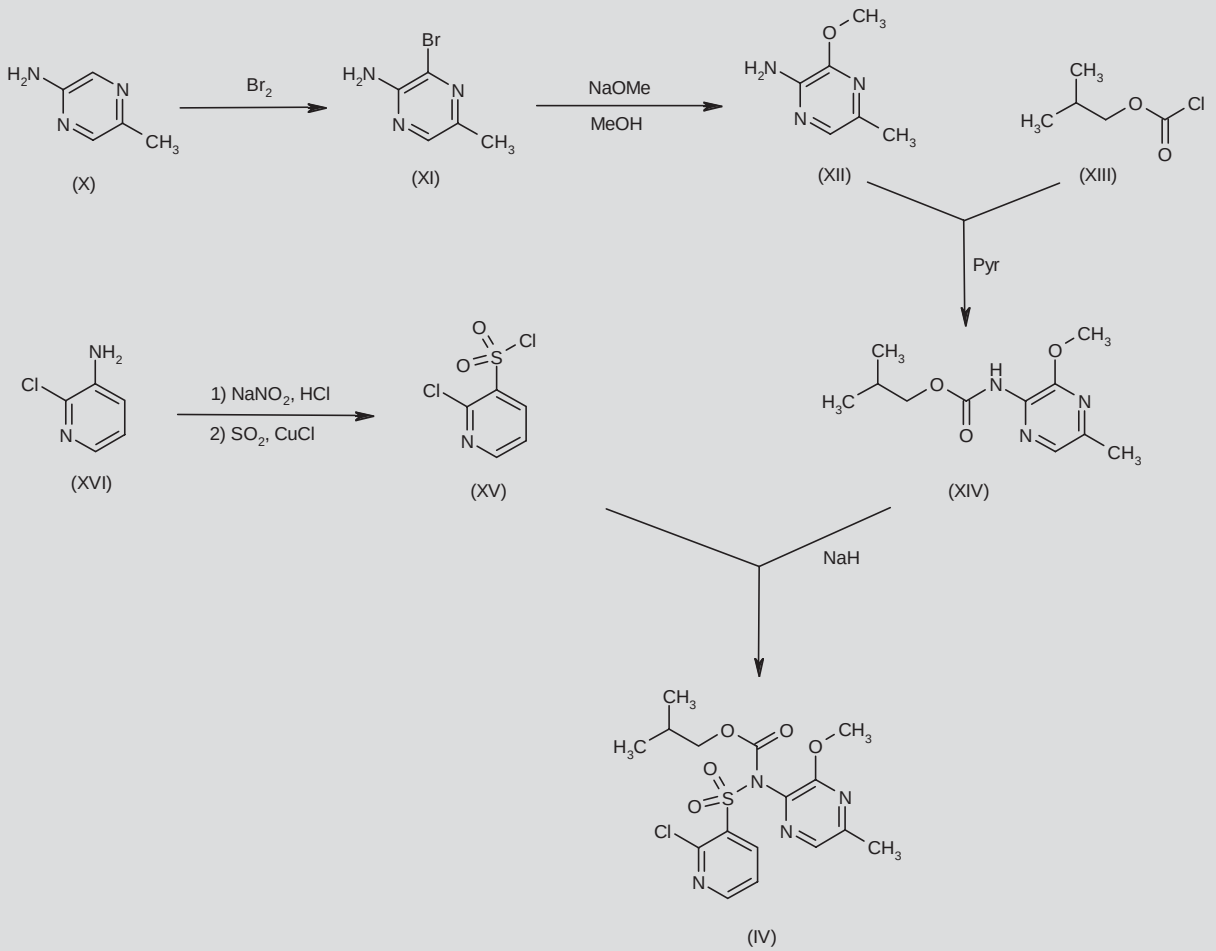
**Synthesis prepared by R. Pandian, J. Bolós, R. Castañer.

Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Zibotentan



Scheme 2. Synthesis of Intermediate (IV)



from 3-amino-2-chloropyridine (XVI) by diazotization and subsequent treatment with sulfur dioxide in the presence of CuCl in AcOH (2). Scheme 2.

BACKGROUND

Prostate cancer is an important health concern across the globe and is the second most common cancer among men (3). Hormone-based therapy for this disease is generally highly effective in the initial stages, but most cases of prostate cancer eventually develop resistance to hormone-based therapies and metastasize, at which point current treatment options become limited (4). An improvement in overall survival has been demonstrated in hormone-resistant prostate cancer (HRPC) with the cytotoxic chemotherapeutic agent docetaxel, although such regimens are associated with significant toxicity and uncertainty as to the appropriate juncture at which to initiate therapy (5-8). Certainly, the lack of a firm consensus over initiation of chemotherapy in this disease area highlights the need for new treatment alternatives that improve survival and maintain the quality of life in patients with HRPC, especially those who are pain-free or only mildly symptomatic.

Endothelin-1 (ET-1) is a 21-amino-acid signaling peptide that regulates various physiological processes, including vasoconstriction, nociception and neural crest development (9, 10). ET-1 is the most potent vasoconstrictor known, being ≥ 10 times more potent than angiotensin II and approximately 100 times more potent than norepinephrine. ET-1 exerts its biological effects via binding to the cell-surface receptors endothelin A (ET_A) and B (ET_B), members of the seven-transmembrane, G protein-coupled receptor family (10). Over the last 15 years, it has emerged that ET-1 plays a key role in the development and progression of advanced prostate cancer (11, 12). While both ET_A and ET_B receptors are expressed and functional in normal prostate tissue, the ET_B receptor appears to be downregulated in prostate cancer cells (13), possibly due to methylation and inactivation of the ET_B receptor gene (14, 15), while expression of the ET_A receptor is increased (16-18). Activation of the ET_A receptor by ET-1 is thought to promote several processes involved in the progression of

prostate cancer, including inhibition of apoptosis, promotion of angiogenesis and invasion, and changes in skeletal biology associated with bone metastasis (19). Conversely, ET_B receptor signaling may have an opposing effect by promoting apoptosis (20) and mediating clearance of ET-1 (21). Antagonism of the ET_B receptor may therefore result in an increase in levels of ET-1, leading to increased ET_A receptor activation and stimulation of tumor progression.

Hence, specific inhibition of the ET_A receptor has potential as a novel treatment approach in HRPC. The selective ET_A receptor antagonist atrasentan has shown positive effects on prostate-specific antigen (PSA) progression and markers of bone disease in patients with HRPC (22). Although atrasentan failed to demonstrate a progression-free survival benefit in recent phase III clinical trials (22, 23), this may have been due to limitations inherent in the design of the trials (24, 25).

Zibotentan (ZD4054) is a specific ET_A receptor antagonist that, unlike atrasentan, has no detectable activity at the ET_B receptor. This class of agent represents a new approach to the treatment of prostate cancer, and this article describes the preclinical and clinical results obtained to date with zibotentan, which support its evaluation in phase III clinical trials.

PRECLINICAL PHARMACOLOGY

Zibotentan is an orally available ET_A receptor antagonist (26-28). In multireceptor binding assays, the pIC_{50} value for zibotentan at the ET_A receptor was 21 nM, while zibotentan had no effect at the ET_B receptor at concentrations up to 100 µM.

Effects of zibotentan on proliferation, apoptosis and invasion in vitro

Consistent with the current understanding of the role of ET-1 in stimulating osteoblasts, zibotentan blocked ET_A-mediated activation of mitogen-activated protein kinase p44/42-MAPK in murine osteoblast cells and inhibited the proliferation of human immature preosteoblast cells (Fig. 1A and B) (29). Moreover, in prostate cancer cell lines, combination of zibotentan with the cytotoxic agents paclitaxel or docetaxel resulted in significant reductions in cell proliferation and increases in apoptosis compared with any agent alone (30). Consistent with its specific binding profile, zibotentan inhibited ET_A receptor-mediated antiapoptotic events while allowing proapoptotic signaling via the ET_B receptor (29). ET-1 inhibited apoptosis induced by serum starvation in rat A10 and human VLTR-16 smooth muscle cell lines, and this effect was reversed by zibotentan in a concentration-responsive manner (Fig. 1C and D). In contrast, selective activation of the ET_B receptor by the peptide agonist BQ-3020 induced proapoptotic signaling in the same cell lines, a response which was not reversed by zibotentan.

In addition to its effects on apoptosis, zibotentan inhibited ET-1-mediated changes in cellular invasiveness. ET-1 induced actin reorganization and phosphorylation of focal adhesion kinase and paxillin in human rhabdomyosarcoma A673 cells, and produced an invasive phenotype, as reflected by three-dimensional invasion into Matrigel (29). These effects of ET-1 were inhibited by zibotentan in a concentration-dependent manner. In human ovarian cancer cells, zibotentan inhibited the epithelial-to-mesenchymal transition that is associated with cancer cell transformation to an invasive phenotype (31). Moreover, in human breast cancer cell lines, combination of zibotentan with aromatase inhibitors or fulvestrant produced at least additive inhibition of migration and invasion (32).

Effects of zibotentan on metastasis, angiogenesis and tumor growth in vivo

Effects of zibotentan on metastasis have been investigated in SCID mice inoculated with a metastasis-selected cell line of human bladder carcinoma (33, 34). While 5 of 9 vehicle-treated mice (55%) developed bone metastases by 6 weeks postinoculation, metastasis was prevented in mice treated with zibotentan in combination with low-dose pamidronate (33). In addition, zibotentan plus pamidronate treatment significantly inhibited the formation of soft tissue metastases in lung and brain (34).

The effects of zibotentan on angiogenesis have been investigated in an intradermal xenograft model of early tumor development. Once-daily treatment for 5 days with zibotentan 25 or 50 mg/kg produced reductions in blood vessel count around the tumor ranging from 15% to 38% versus control in seven separate experiments. These reductions, although moderate, were consistent across the xenograft models tested, which were derived from human prostate adenocarcinoma PC-3, LoVo, and DU 145 cancer cell lines. In addition, zibotentan reversed the dilatation of normal blood vessels that occurs around developing tumors. This reversal of tumor-induced vasodilatation was an unexpected finding given that ET_A receptor antagonism in itself would be expected to promote vasodilatation. It may be that zibotentan has antagonist efficacy against a vasodilatory factor produced by the tumor, and is thus able to induce reversal of vasodilatation in an indirect manner (35). A link between the profoundly vasodilatory growth factor vascular endothelial growth factor (VEGF) and the ET axis has been previously proposed (36-38).

In addition to inhibiting metastasis and angiogenesis in the preclinical setting, zibotentan significantly inhibited the growth of primary tumor xenografts. Zibotentan 25 mg/kg/day inhibited the growth of s.c. human ovarian cancer HEY xenografts in nude mice, and this growth inhibition was comparable to that achieved by paclitaxel (three doses of 20 mg/kg i.v. every 4 days). Moreover, in combination, zibotentan and paclitaxel produced significantly greater growth inhibition than that seen with either agent alone (39). Likewise, the combination of zibotentan with the epidermal growth factor receptor (EGFR) inhibitor gefitinib (Iressa™; AstraZeneca) produced greater growth-inhibitory effects than either agent alone (40). These results add to the accumulating body of preclinical evidence that supports the strategy of cotargeting multiple pathways in the treatment of cancer.

PHARMACOKINETICS AND METABOLISM

Metabolism studies showed that [^{14}C]-labeled zibotentan was rapidly absorbed, with a t_{max} of approximately 1 h, and that excretion was predominantly via the urine (71-94% of the dose) (41). The terminal elimination half-life was approximately 8 h. Zibotentan exhibited a moderate level of plasma protein binding (mean 73.8%), which suggests that pharmacokinetic parameters are unlikely to be affected by protein binding displacement interactions upon comedication with

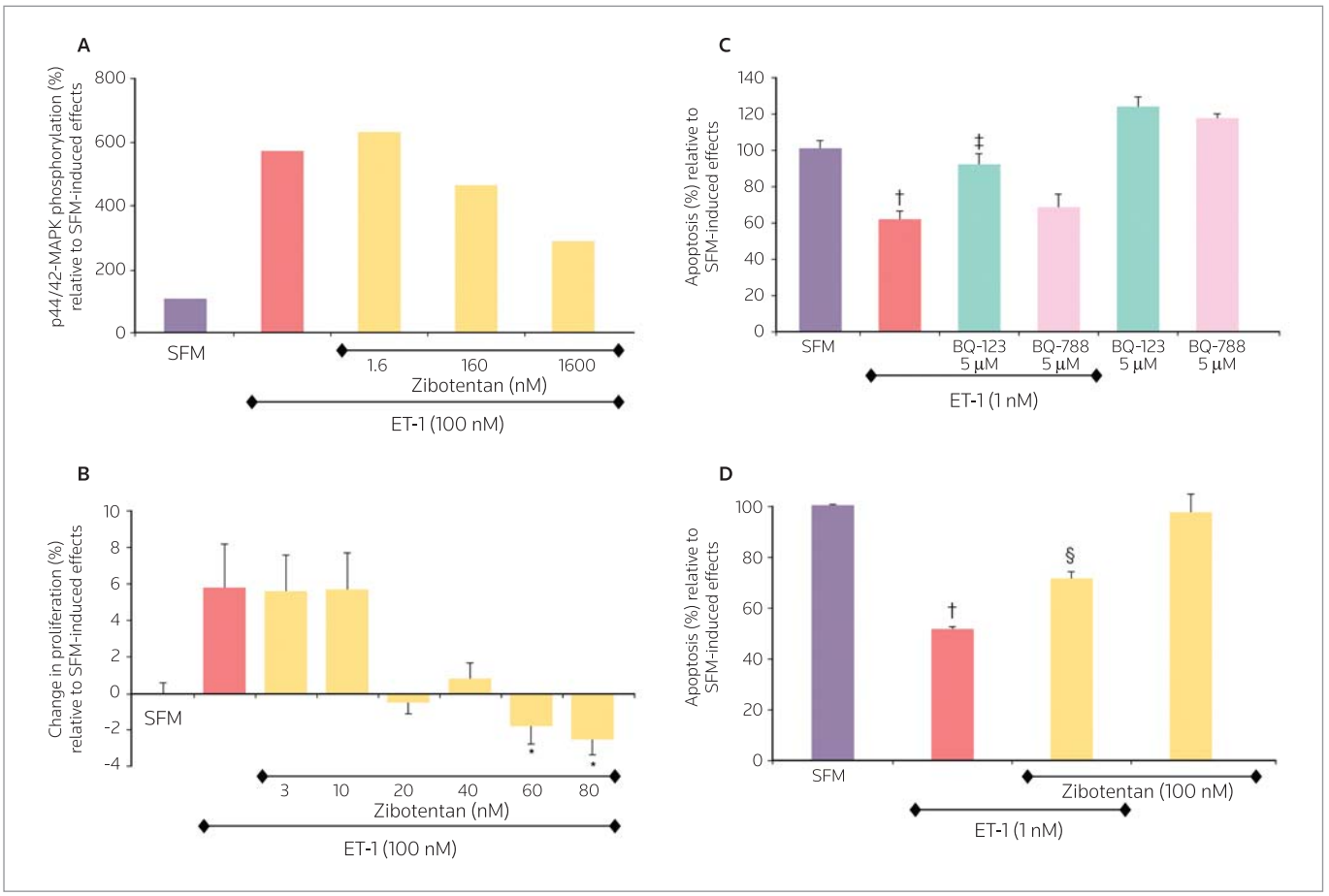


Figure 1. Effects of zibotentan on proliferation and mitogen-activated protein kinase p44/42-MAPK phosphorylation in serum-deprived HBC-171 cells (**A** and **B**), and effects of ET-1 and ET_A and ET_B receptor antagonists on apoptosis in serum-deprived VLTR-16 cells (**C** and **D**). Zibotentan concentration-dependently inhibited (A) ET-1-induced p44/42-MAPK phosphorylation and (B) ET-1-induced proliferation. The histograms represent the mean percent relative change in viable cells (compared with the effect seen in serum-free media [SFM]) obtained from at least three independent experiments, and bars are SEM. *$P < 0.05$ vs. SFM. (C) ET_A receptor inhibition with BQ-123, but not ET_B receptor inhibition with BQ-788, reverses ET-1-mediated inhibition of apoptosis induced by serum deprivation. (D) zibotentan concentration-dependently reverses ET-1-mediated inhibition of apoptosis induced by serum deprivation. The histograms represent the mean percent apoptosis relative to SFM-induced effects obtained from at least three independent experiments, and bars are SEM. †$P < 0.05$ vs. SFM; ‡$P < 0.01$ and §$P < 0.05$ vs. ET-1. Reprinted from Anti-Cancer Drugs, 20(2), Growcott, J. *Preclinical anticancer activity of the specific endothelin A receptor antagonist ZD4054*, pp. 83-8, © 2009, with permission from Wolters Kluwer Health.

other drugs. A comparison of zibotentan in Caucasian and Japanese patients with HRPC treated with doses of 5, 10 and 15 mg found that pharmacokinetic parameters were similar between the two populations. Zibotentan was rapidly absorbed and the mean half-life ranged between 9 and 12 h. Little accumulation was observed after repeated once-daily oral dosing, and multiple-dose pharmacokinetics were predictable (42).

SAFETY

Single oral doses of zibotentan of up to 120 mg were well tolerated in healthy volunteers, with dose escalation limited by headache, nausea and vomiting (28). An open-label, multicenter, multiple-ascending-dose phase I study investigated the tolerability of zibotentan in patients with HRPC (43). A total of 16 patients received once-daily oral tablets of zibotentan at doses of 10 (n = 3), 15 (n = 9) and 22.5 mg (n = 4). Dose-limiting toxicities at the dose of 22.5 mg were dyspnea and peripheral edema in one patient and grade 3 headache and intraventricular hemorrhage in a second patient. No dose-limiting toxicities were seen at the dose of 15 mg, and the most frequent adverse events at this dose were headache, peripheral edema, fatigue, nasal congestion and nausea. This tolerability profile is consistent with the known pharmacological effect of ET_A receptor antagonism.

CLINICAL STUDIES

Specificity for the ET_A receptor

Functional antagonism of the ET_A receptor by zibotentan in humans was assessed by measuring vasoconstriction in healthy male volunteers (28). ET-1 causes vasoconstriction through activation of ET_A receptors on vascular smooth muscle (44), so ET_A receptor blockade would be expected to inhibit ET-1-induced vasoconstriction. Follow-

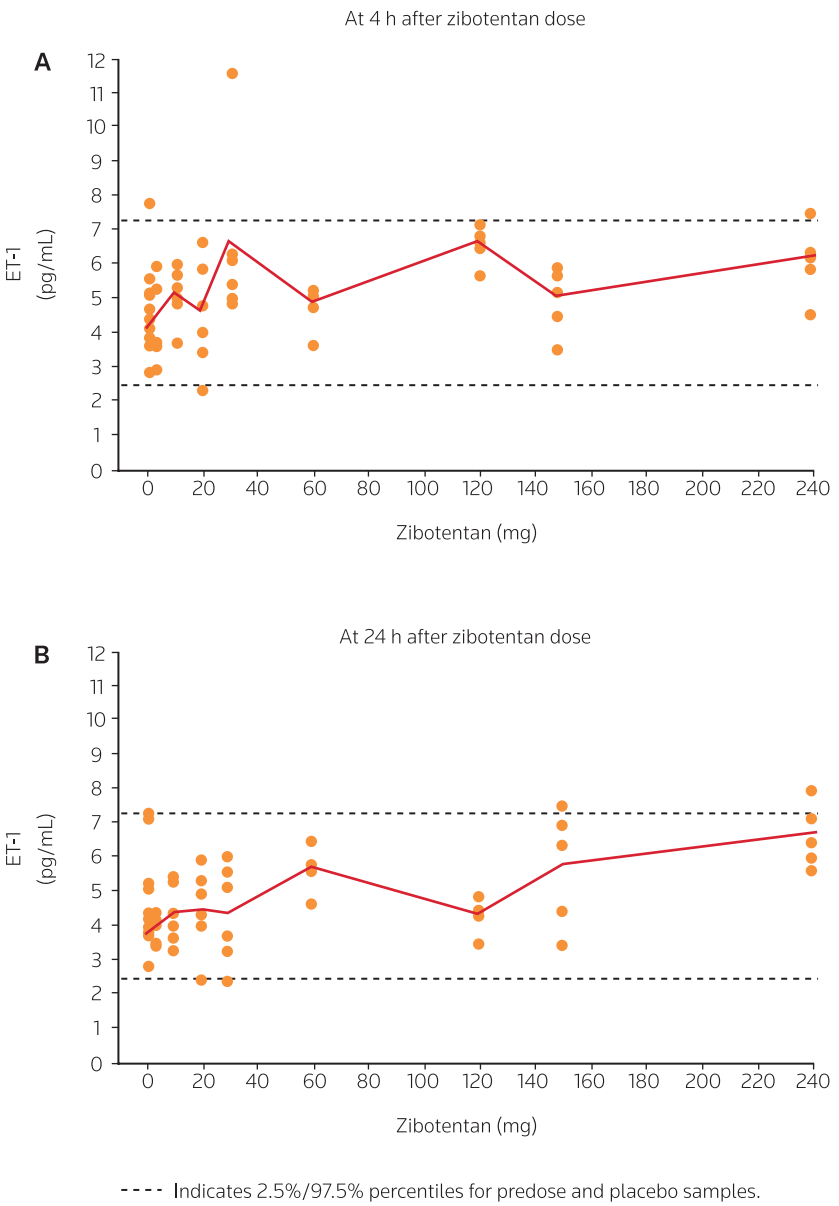


Figure 2. Zibotentan has no effect on ET-1 levels in healthy volunteers. Administration of zibotentan at doses up to 240 mg had no effect on plasma ET-1 concentrations in healthy volunteers at (**A**) 4 h and (**B**) 24 h after dosing. Individual and mean data are shown. Reprinted from Br J Cancer, 92(12), Morris, C.D., Rose, A., Curwen, J., Hughes, A.M., Wilson, D.J., Webb, D.J. *Specific inhibition of the endothelin A receptor with ZD4054: Clinical and pre-clinical evidence*, pp. 2148-52, © 2005, with permission from Macmillan Publishers Ltd.

ing a 120-min brachial artery infusion of ET-1 (2.5 pmol/min), single oral doses of zibotentan of 10 and 30 mg reduced forearm vasoconstriction, measured by venous occlusion plethysmography, by 38% and 63%, respectively, compared with placebo. These results confirm that zibotentan antagonizes the ET_A receptor in humans.

The specificity of zibotentan for the ET_A receptor relative to the ET_B receptor was demonstrated by measuring levels of circulating ET-1. The ET_B receptor clears ET-1 from the circulation, and ET_B receptor antagonism increases circulating levels of ET-1 (19, 45). The selective ET_A receptor antagonist atrasentan increased levels of circulating ET-1 in clinical trials in a dose-dependent manner, which may be attributed to its low-level antagonism of the ET_B receptor (46, 47). In contrast, zibotentan produced no increase in levels of circulating ET-1 after single oral doses up to 240 mg (Fig. 2), confirming the absence of zibotentan activity at the ET_B receptor (28).

Recent and ongoing clinical trials

The results of early clinical trials supported a large phase II trial in men with HRPC. A randomized, double-blind, placebo-controlled, parallel-group phase II trial (the EPOC study; Endothelin A receptor antagonism Proof Of Concept) was undertaken at 65 centers in 14 countries across Europe, North America, Australasia and South East Asia (48). A total of 312 patients with HRPC and bone metastases who were pain-free or mildly symptomatic for pain were recruited and randomized to receive once-daily zibotentan 10 mg (n = 107) or 15 mg (n = 98), or matching placebo (n = 107). The primary endpoint was time to progression, defined as clinical progression, requirement for opiate analgesia, objective progression of soft tissue metastases or death in the absence of progression. PSA progression and change in the number or appearance of bone metastases on scintigraphic imaging did not count as progression events. Secondary endpoints included overall survival, PSA progression and safety. At the primary analysis, no statistically significant difference in time to progression was observed for zibotentan versus placebo (hazard ratio [HR]: 0.88 [80% confidence interval (CI): 0.71-1.09] for 10 mg; HR: 0.83 [80% CI: 0.66-1.03] for 15 mg; Fig. 3). However, a promising signal for prolonged overall survival was observed in the zibotentan treatment groups versus placebo based on 40 deaths

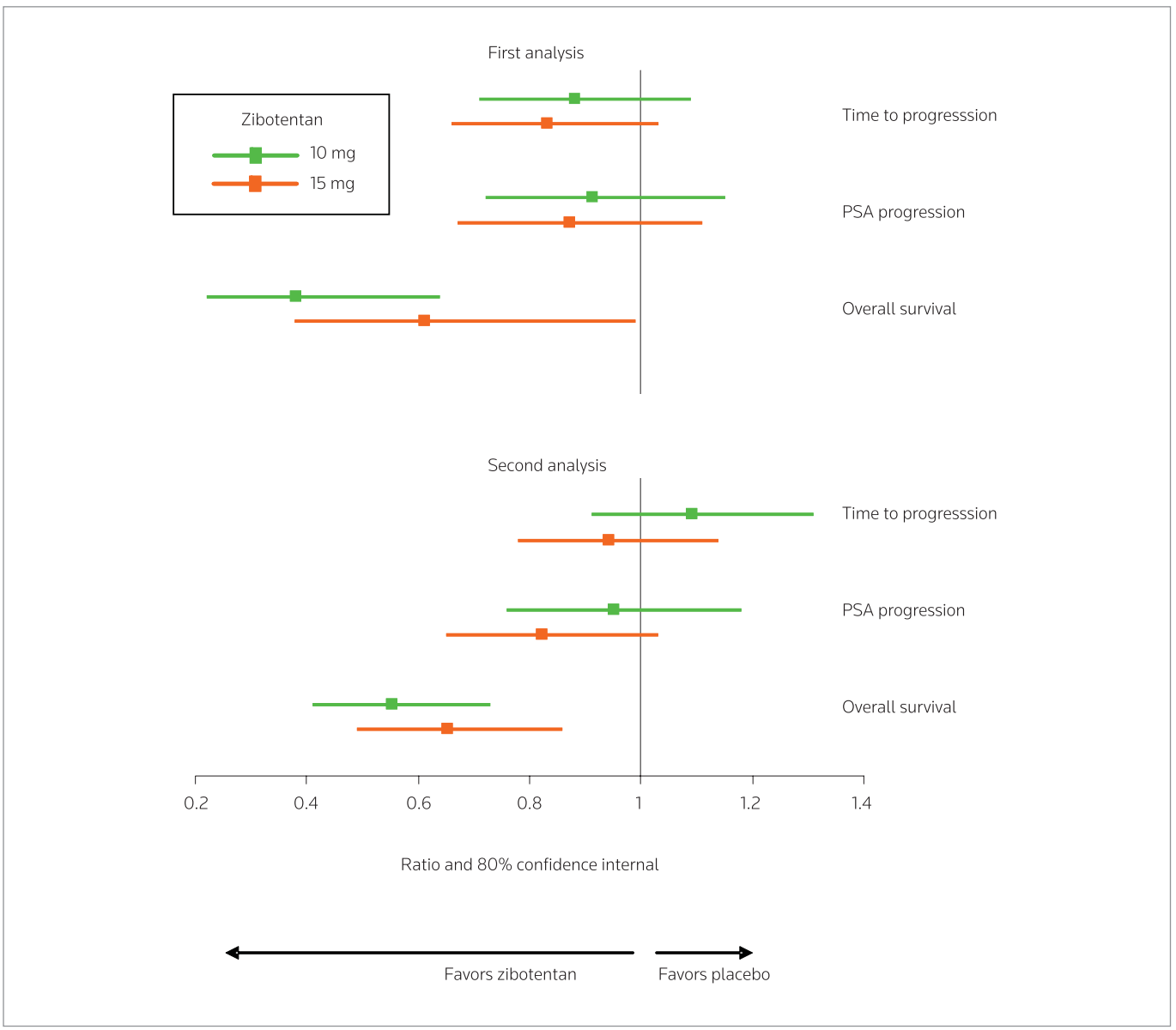


Figure 3. Hazard ratio versus placebo and confidence intervals for key endpoints of the zibotentan phase II trial (first analysis after 40 deaths and second analysis after 118 deaths). Reprinted from Eur Urol, 55(5), James, N.D., Caty, A., Borre, M. et al. *Safety and efficacy of the specific endothelin A receptor antagonist ZD4054 in patients with hormone-resistant prostate cancer and bone metastases who were pain free or mildly symptomatic: A double-blind, placebo-controlled, randomized, phase II trial*, pp. 1112-23, © 2009, with permission from Elsevier.

(HR: 0.38 [80% CI: 0.22-0.64] for 10 mg zibotentan, $P = 0.019$; HR: 0.61 [80% CI: 0.38-0.99] for 15 mg zibotentan, $P = 0.190$). At the second analysis, after 118 deaths, the survival benefit was sustained (HR: 0.55 [80% CI: 0.41-0.73] for 10 mg zibotentan, $P = 0.008$; HR: 0.65 [80% CI: 0.49-0.86] for 15 mg zibotentan, $P = 0.052$), while there continued to be no significant difference in time to progression. Median overall survival was 24.5 and 23.5 months, respectively, in the zibotentan 10 and 15 mg groups compared with 17.3 months in the placebo group. No significant differences were observed in time to PSA progression. Adverse events were in line with the expected pharmacodynamic effects of an ET_A receptor antagonist, most commonly headache, peripheral edema and nasal congestion.

The discordance observed between results for the primary endpoint, time to progression, and overall survival may reflect the sensitivity of the definition used for progression. This definition resulted in more than 35% of patients experiencing a progression event within 3 months of randomization. Significant treatment benefits that emerge over long time scales may not be reflected in composite definitions of progression that are sensitive to relatively minor changes in prostate cancer symptoms.

The promising improvement in overall survival with zibotentan seen in the EPOC study supports further investigation in phase III clinical trials, with overall survival as the primary endpoint. The zibotentan ENdoTHelin A inhibitor USE (ENTHUSE) phase III clinical trial program consists of three randomized, double-blind trials, which together will include more than 3,000 patients with HRPC across more than 400 centers worldwide. In each trial, zibotentan will be administered as a once-daily dose of 10 mg; this dose level has been selected because the phase II EPOC results showed no improved efficacy for the 15-mg dose compared with the 10-mg dose.

In ENTHUSE M0 (ClinicalTrials.gov identifier NCT00626548), 1,500 patients with M0 HRPC (rising PSA but no evidence of metastatic spread) will be randomized in a 1:1 ratio to zibotentan or placebo. In addition to the primary endpoint of overall survival, ENTHUSE M0 has a coprimary endpoint of progression-free survival, defined as the time to appearance of metastases. Secondary endpoints of this trial include safety and tolerability, PSA and health-related quality of life. The ENTHUSE M0 study is expected to run for approximately 5 years.

ENTHUSE M1 (ClinicalTrials.gov identifier NCT00554229) is similar to the phase II EPOC study (48), investigating zibotentan in 580 patients with M1 HRPC (bone metastases and rising serum PSA despite medical or surgical castration). Patients in this trial have been randomized 1:1 to zibotentan or placebo, with a primary study endpoint of overall survival. Secondary endpoints include progression-free survival, safety and tolerability, skeletal events and bone metastases, PSA and health-related quality of life. The total duration of the ENTHUSE M1 study is expected to be 30 months.

The third trial, ENTHUSE M1c (ClinicalTrials.gov identifier NCT00617669), will randomize 1,044 patients with confirmed metastatic HRPC suitable for chemotherapy to receive zibotentan in combination with docetaxel or placebo in combination with docetaxel. The primary endpoint of ENTHUSE M1c is overall survival. Secondary endpoints of this trial include progression-free survival, PSA, safety and tolerability, and the effect of treatment on skeletal events. The duration of the ENTHUSE M1c study is expected to be 36 months.

The ENTHUSE trial program will investigate the use of zibotentan in a real-world clinical setting, with the drug or placebo being used in addition to existing care. As part of this, other therapies used in the management of prostate cancer, such as symptomatic pain control, treatment of urinary obstruction, bisphosphonate, steroid and secondary hormonal therapies, may be given to patients at the investigator's discretion without trial therapy being halted. This approach accords with the recommendations of the Prostate Cancer Clinical Trials Working Group (PCWG2) (49), and should help avoid the limitations of recent HRPC trials in which study drug was discontinued prematurely as PSA levels rose (25).

DRUG INTERACTIONS

As zibotentan is metabolized by the cytochrome P450 system via isozyme 3A4 (CYP3A4), the possibility of drug interactions with the cytochrome P450 inducer rifampicin and the CYP3A4 inhibitor itraconazole was investigated (50). Rifampicin coadministration reduced zibotentan exposure (AUC) after a single dose of 15 mg zibotentan by a mean of 68%, from a geometric mean of 7640 ng·h/mL (coefficient of variance [CV]: 32%) to 2444 ng·h/mL (CV: 15%). As this could potentially reduce the clinical efficacy of zibotentan, concomitant use of potent CYP3A4 inducers should be avoided. Itraconazole coadministration increased zibotentan AUC after a single 10-mg dose of zibotentan by 27%, from a mean of 3195 ng·h/mL (CV: 22%) to 4045 ng·h/mL (CV: 27%), an effect that is unlikely to significantly alter the safety profile.

CONCLUSION

The specific ET_A receptor antagonist zibotentan demonstrated encouraging anticancer effects in preclinical studies, consistent with the current scientific understanding of the role of the ET_A receptor in the development and progression of cancer. In clinical studies once-daily oral zibotentan was generally well tolerated in patients with HRPC, having an adverse effect profile consistent with its known pharmacological activity. A promising improvement in overall survival was observed in the phase II EPOC study, suggesting that zibotentan may have therapeutic benefit in patients with HRPC. These results endorse the strategy of targeting the ET_A receptor in prostate cancer, and support further investigation of zibotentan in larger phase III clinical trials. The zibotentan ENTHUSE phase III clinical trial program is currently under way, with overall survival as primary endpoint.

SOURCE

AstraZeneca (GB).

DISCLOSURE

NJ has received honoraria and consultancy fees from AstraZeneca, Lilly, Bayer, Pfizer, Amgen, Novartis and various other companies, and grant support in respect of costs of clinical trials from various companies, including AstraZeneca. JG is an employee of AstraZeneca. Editorial assistance was provided by Matt Lewis, PhD, of Mudskipper Bioscience, on behalf of AstraZeneca.

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