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MONOGRAPH

SARACATINIB

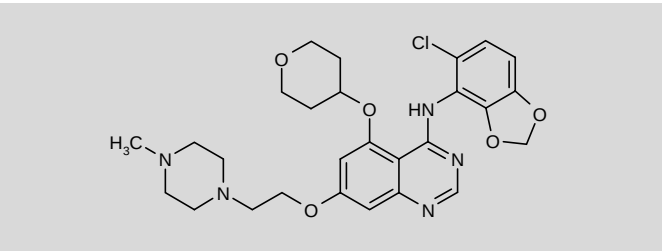
Prop INN

Dual Src/ABL Kinase Inhibitor
Oncolytic

AZD-0530

N-(5-Chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine

InChI=1/C27H32ClN5O5/c1-32-6-8-33(9-7-32)10-13-35-19-14-21-24(23(15-19)38-18-4-11-34-12-5-18)27(30-16-29-21)31-25-20(28)2-3-22-26(25)37-17-36-22/h2-3,14-16,18H,4-13,17H2,1H3,(H,29,30,31)



$C_{27}H_{32}ClN_5O_5$
Mol wt: 542.026
CAS: 379231-04-6
EN: 323895

ABSTRACT

Tumor development is usually triggered by abnormalities in complex signaling pathways involved in normal cell growth, activity and function. Src kinase has been identified as a critical signaling pathway involved in tumor cell migration and invasion in multiple cancers, therefore representing a promising pharmacotherapeutic target. To date, no inhibitor of Src kinase has been granted marketing approval. Saracatinib (AZD-0530) is an oral, highly selective dual inhibitor of the Src and ABL kinases. This monograph highlights the preclinical and clinical studies completed to date for saracatinib, which is currently in phase II clinical development for ovarian, breast, prostate, colorectal, lung, bone, pancreatic, skin, gastric, thymic and head and neck cancers.

SYNTHESIS

Saracatinib can be prepared by several different ways:

Condensation of 7-benzyloxy-4-chloro-5-(tetrahydropyran-4-yloxy)quinazole (I) with 5-chloro-1,3-benzodioxol-4-amine (II) by means of HCl in isopropanol gives the secondary amine (III), which is debenzylated by means of H_2 over Pd/C in ethanol/THF to yield the 7-hydroxyquinazoline derivative (IV). Finally, this compound is condensed with 1-(2-hydroxyethyl)-4-methylpiperazine (V) by means of DBAD and PPh_3 in dichloromethane (1). Scheme 1.

Reaction of 2,4,6-trifluorobenzonitrile (VI) with ammonia in hot isopropanol gives 2-amino-4,6-difluorobenzonitrile (VII), which is treated with hot aqueous H_2SO_4 to afford 2-amino-4,6-difluorobenzamide (VIII). Cyclization of benzamide (VIII) with triethyl orthoformate by means of HCl at 140 °C affords 5,7-difluoroquinazolin-4(3*H*)-one (IX), which is condensed with 4-hydroxytetrahydropyran (X) by means of *t*-BuOK in refluxing THF to provide the 5-(tetrahydropyran-4-yloxy)quinazoline derivative (XI). Condensation of compound (XI) with 1-(2-hydroxyethyl)-4-methylpiperazine (V) by means of *t*-BuOK in refluxing THF gives the disubstituted quinazolinone (XII), which is finally condensed with 6-chloro-2,3-methylenedioxyaniline (II) by means of $POCl_3$ in refluxing acetonitrile (2). Scheme 2.

Alternatively, difluoroquinazolinone (IX) is treated with $POCl_3$ and DIEA in hot acetonitrile to give 4-chloro-5,7-difluoroquinazoline (XIII), which is condensed with 5-chloro-1,3-benzodioxol-4-amine (II) by means of DIEA in hot acetonitrile to yield the 4-amino-substituted quinazoline (XIV). Reaction of compound (XIV) with 4-hydroxytetrahydropyran (X) by means of *t*-BuOK in refluxing THF affords the 5-(tetrahydropyranyloxy)quinazoline derivative (XV), which is finally condensed with 1-(2-hydroxyethyl)-4-methylpiperazine (V) by means of KOH in di(2-methoxyethyl)ether at 120 °C (2). Scheme 2.

Esterification of 2-amino-4,6-dimethoxybenzoic acid (XVI) with diazomethane in ethanol gives the corresponding methyl ester (XVII), which is cyclized with formamidine (XVIII) in refluxing 2-methoxyethanol to yield 5,7-dimethoxyquinazolin-4(3*H*)-one (IXX). Selective demethylation of compound (IXX) by means of $MgBr_2$ in refluxing pyridine affords 5-hydroxy-7-methoxyquinazolin-4(3*H*)-one (XX), which is condensed with pivaloyloxymethyl chloride (POM-Cl) and NaH in DMF to provide the protected quinazolinone (XXI). Condensation of compound (XXI) with 4-hydroxytetrahydropyran (X) by means of PPh_3 in dichloromethane gives 7-methoxy-5-(tetrahydropyran-4-yloxy)quinazolin-4(3*H*)-one (XXII), which is demethylated by means of PhSH and K_2CO_3 in NMP at 195 °C to yield the 7-hydroxyquinazolinone derivative (XXIII). Acylation of compound (XXIII) with acetic anhydride affords the 7-acetoxy derivative (XXIV) , which is treated with $POCl_3$ at 80 °C to provide 7-acetoxy-4-chloro-

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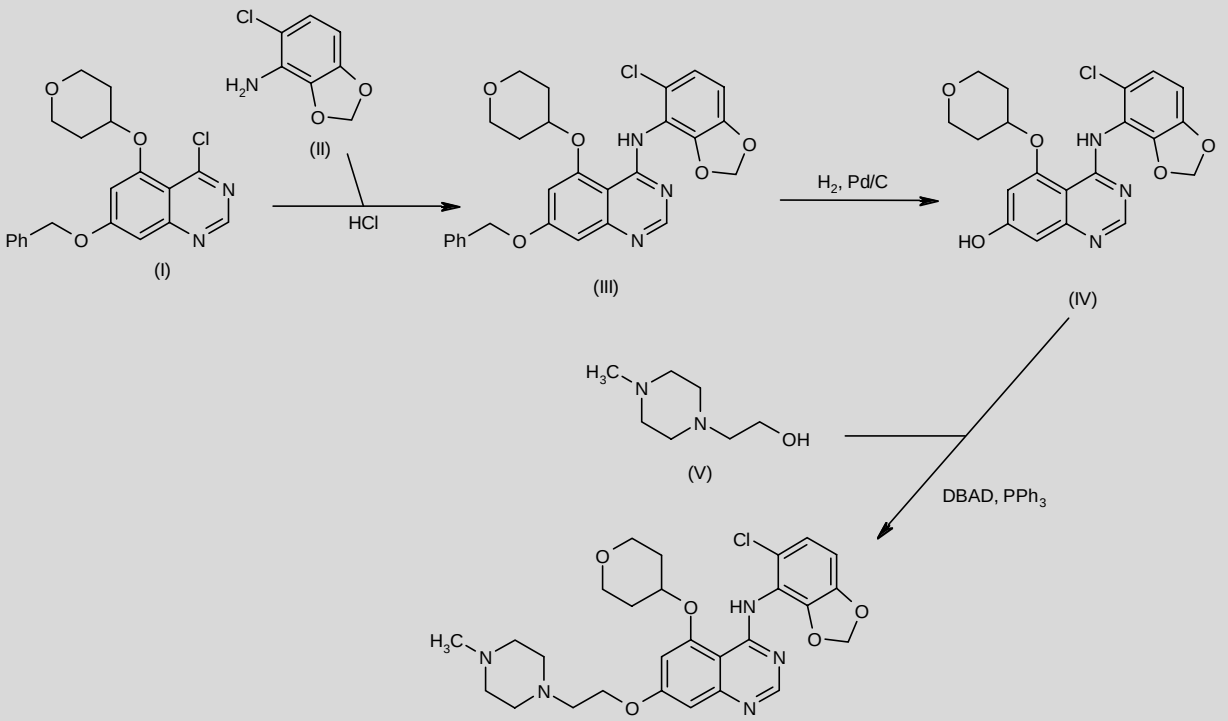
Scheme 1. Synthesis of Saracatinib



5-(tetrahydropyran-4-yloxy)quinazoline (XXV). Condensation of (XXV) with 4-amino-5-chloro-1,3-benzodioxole (II) in hot isopropanol gives adduct (XXVI), which is treated with ammonia in methanol to afford the 7-hydroxyquinazoline derivative (IV). Finally, this compound is condensed with 1-(2-chloroethyl)-4-methylpiperazine (XXVII) by means of K_2CO_3 in DMF (3, 4). Scheme 3.

BACKGROUND

Cancer is a growing concern worldwide (5, 6); the World Health Organization (WHO) has indicated that cancer causes approximately 13% of all deaths (7). Therapies targeting deregulated proteins specific to cancer cells have been emerging since the late 1990s and represent a promising treatment avenue for multiple types of cancer.

c-Src is a 60-kDa nonreceptor tyrosine kinase encoded by the *SRC* gene and is the cellular homologue to the potent transforming v-Src viral oncoprotein. c-Src functions in an array of signal transduction cascades that influence cellular proliferation, differentiation, motility and survival. It is highly regulated and active only at low levels in normal cells, whereas studies in many human tumor types have indicated that c-Src is upregulated (8, 9). Consequently, c-Src has emerged as an interesting therapeutic target for multiple cancers.

Saracatinib (AZD-0530) is a highly selective, orally available small-molecule inhibitor of Src kinase (IC_{50} = 2.7 nM) and the related ABL kinase (IC_{50} = 30 nM) under development by AstraZeneca (4, 10). Saracatinib has reached phase II clinical trials; studies currently active with this agent are outlined in Table I.

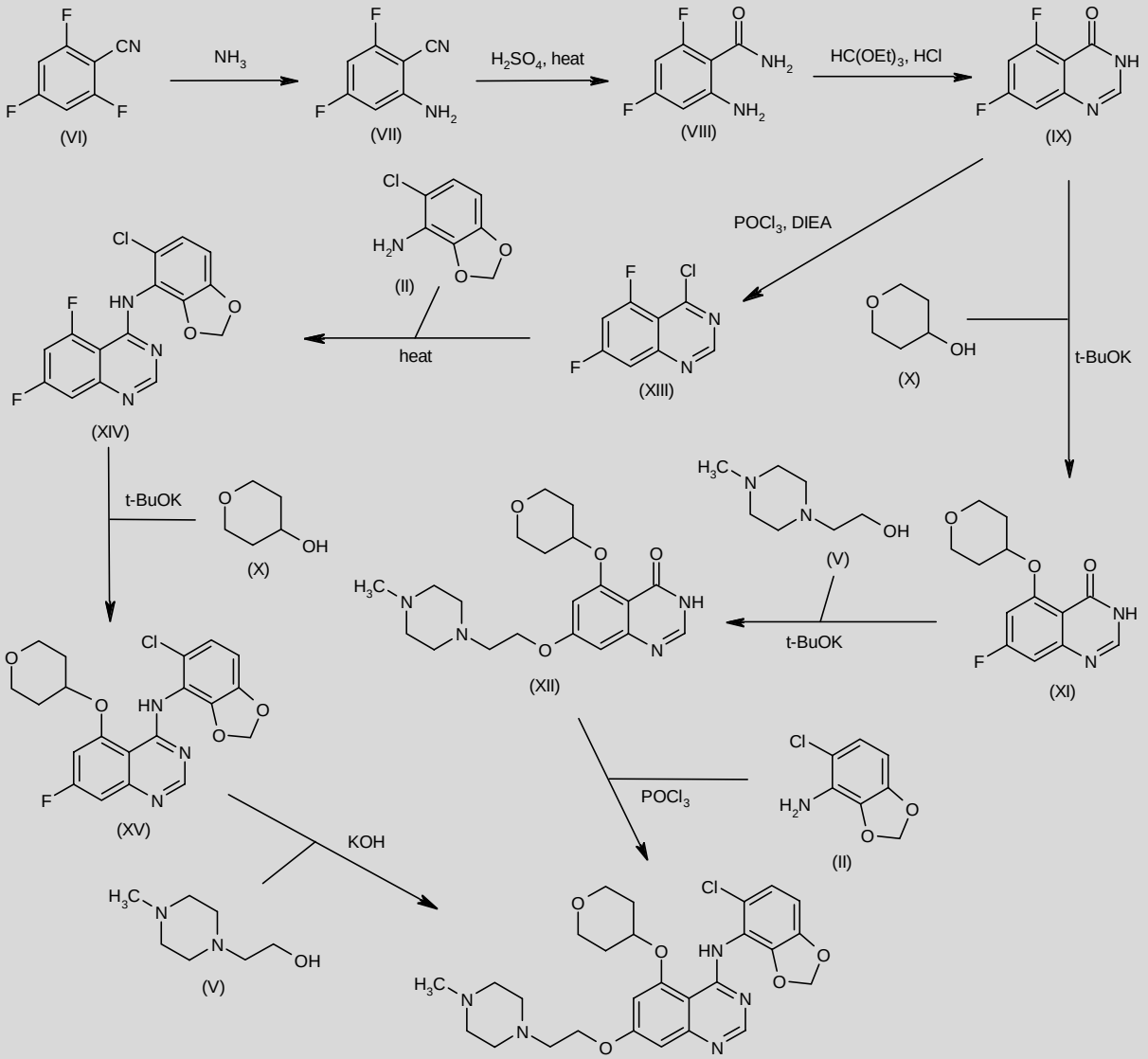
PRECLINICAL PHARMACOLOGY

In vitro investigations have confirmed that saracatinib can induce apoptosis and cell cycle arrest in the lymphoma cell lines DOHH-2 and WSU-NHL. Apoptosis observed in these cell-based assays was shown to be caspase-dependent, with downregulation of the anti-apoptotic protein Bcl-XL (11).

Saracatinib has shown activity against tamoxifen-resistant human breast cancer MCF7 cells (IC_{50} = 0.47 µmol/L). Further studies have also demonstrated successful saracatinib-mediated inhibition of anchorage-dependent growth in MCF7 cells overexpressing the mutant K303 estrogen receptor ERα, which has been shown to promote breast tumor growth, with an IC_{50} of 1.28 µmol/L. These cells were shown to be more sensitive to growth inhibition with coadministration of tamoxifen and saracatinib (12, 13). Coapplication of saracatinib with the aromatase inhibitor anastrozole to MCF7 cells also demonstrated synergistic growth inhibition (14).

Saracatinib has demonstrated efficacy in in vitro studies in head and neck squamous cell carcinoma (HNSCC) cell lines. MTT cell viability assays in 1483, HN31 and UMSCC19 cells have shown that saracatinib (0.1 µM) inhibits HNSCC motility and invasion in transwell assays, mediated by the formation of invadopodia (protrusions arising from the ventral cell surface that focally degrade extracellular matrix) (15).

Scheme 2. Synthesis of Saracatinib



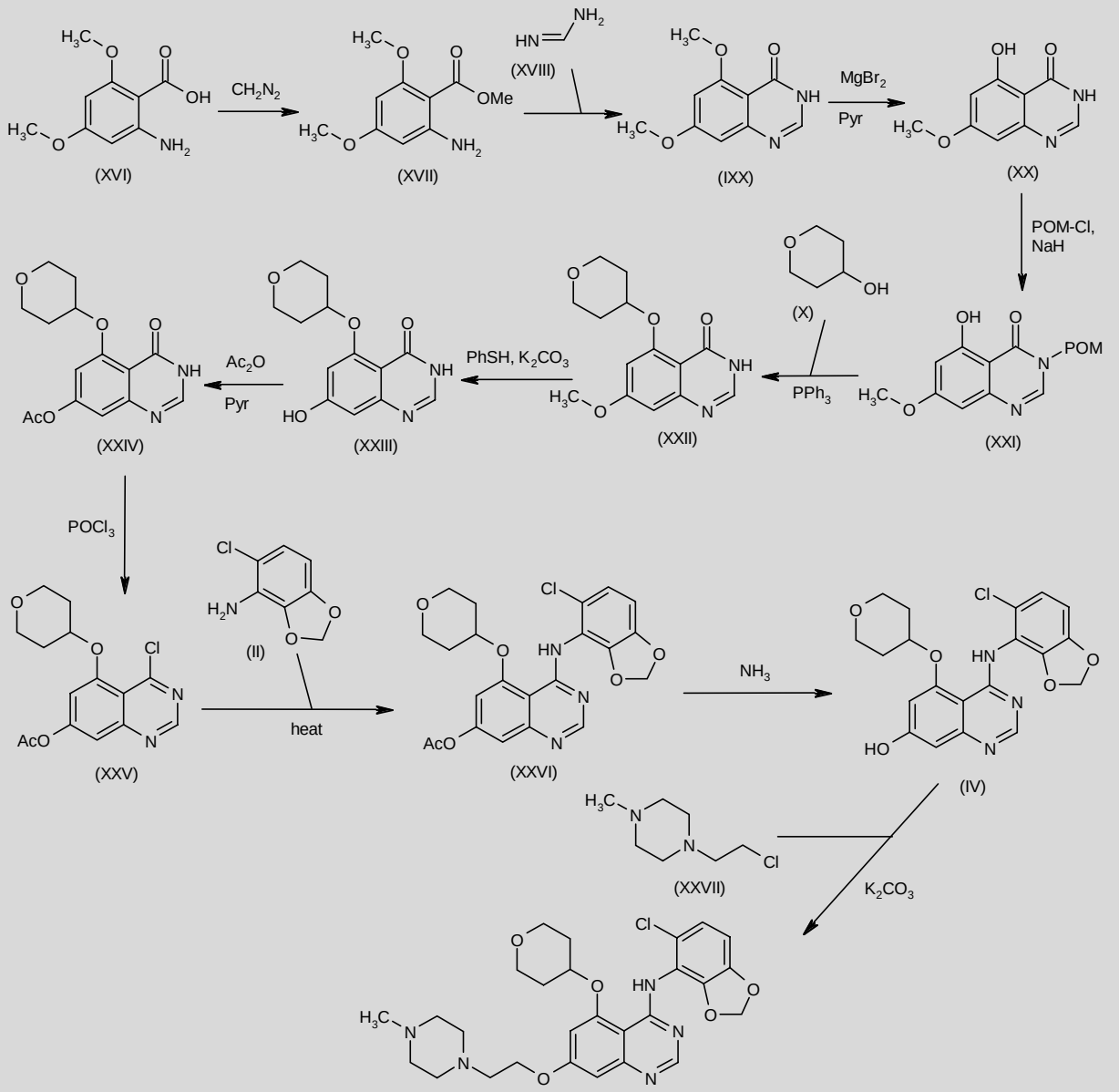
Studies in HNSCC cell lines expressing a dominant-active form of c-Src and showing increased growth and invasion also demonstrated the effectiveness of combined targeting of c-Src and epidermal growth factor receptor (EGFR). IC_{50} values for saracatinib in HNSCC cell lines 1483, UM-22B, PCI-15B, PCI-37B and Cal-33 were reported to be 1, 1, 1.3, 1 and 0.6 μmol/L, respectively. Combined application of saracatinib and the EGFR inhibitor gefitinib facilitated a greater inhibition of HNSCC cell growth and invasion compared with either agent alone (16). Coapplication of saracatinib and erlotinib, another EGFR inhibitor, also demonstrated antitumor effects in HNSCC cell lines (17). Further studies have confirmed that combined inhibition of c-Src and phospholipase C-γ-1 (PLC-γ-1) via the coapplication of saracatinib and U-73122 provides enhanced abrogation of HNSCC cell invasion (18).

Additional studies have confirmed that saracatinib-mediated growth inhibition in HNSCC cell lines is more effective in epithelial versus mesenchymal lines (IC_{50} < 1 μM vs. > 7 μM). Despite this, saracatinib-mediated inhibition of migration and invasion is not restricted to specific cell lines (19).

Studies in human rhabdomyosarcoma A673 tumor cell lines showed enhanced in vitro anti-invasive activity with the combination of saracatinib and the specific endothelin A receptor (ET_A) antagonist ZD-4054. Synergistic anti-invasive activity with a combination index of < 0.85 was reported (20, 21).

In vitro studies in human ovarian carcinoma A2780 cells expressing the *MUC16* oncogene (expressed in approximately 80% of ovarian

Scheme 3. Synthesis of Saracatinib



malignancies) have shown that saracatinib reduces the invasion of these cells by 89% (22).

Investigations in prostate cancer cell lines (CWR22Rv1, DU 145, LNCaP and PC-3) have shown that saracatinib inhibits cell proliferation via G_0/G_1 cell cycle arrest, with further evidence to suggest downregulation of the transcription factor c-Myc (23). Further studies have shown that saracatinib inhibits the growth of PC-3 cells in vitro with an associated reduction in factors that are generally overexpressed in prostate cancer (focal adhesion kinase [FAK] and phosphorylated paxillin) (24).

The effect of saracatinib on bone metastasis has been tested using human co-culture systems. Saracatinib (0.1-10 μM) successfully inhibited the formation of multinucleated osteoclast-like cells, in a concentration-dependent manner. Further investigation confirmed that this effect of saracatinib was most probable during the initial induction of osteoclast formation (osteoclastogenesis) and that saracatinib prevented the migration of osteoclast precursors to the bone surface and the subsequent formation of actin rings (to which c-Src co-localizes and which is a prerequisite for osteoclastic bone resorption) (25, 26).

Table I. *Currently active clinical trials for saracatinib.*

Phase	Condition	Source	Ref.
II/III	Healthy volunteers	AstraZeneca	43
II	Ovarian cancer	AstraZeneca	44
	Breast cancer Prostate cancer Bone neoplasms	AstraZeneca	45
	Osteosarcoma	Sarcoma Alliance for Research through Collaboration/AstraZeneca	46
	Breast cancer	Memorial Sloan-Kettering Cancer Center/National Cancer Institute (NCI)	47
	Pancreatic cancer	Mayo Clinic/National Cancer Institute (NCI)	48
	Endometrial cancer, Sarcoma	Princess Margaret Hospital/National Cancer Institute (NCI)	49
	Melanoma (skin)	University of Chicago/National Cancer Institute (NCI)	50
	Thymoma, thymic carcinoma	Indiana University Melvin and Bren Simon Cancer Center/National Cancer Institute (NCI)	51
	Gastric cancer	Princess Margaret Hospital/National Cancer Institute (NCI)	52
	Head and neck cancer	Memorial Sloan-Kettering Cancer Center/National Cancer Institute (NCI)	53
	Lung cancer	Princess Margaret Hospital/North Central Cancer Treatment Group/National Cancer Institute (NCI)	54, 55
	Colon cancer	M.D. Anderson Cancer Center/National Cancer Institute (NCI)	56
I	Solid tumors	AstraZeneca	57-59
	Healthy volunteers	AstraZeneca	60

In vitro exposure of human HOS-MNNG osteosarcoma cells to saracatinib at noncytotoxic concentrations as low as 0.1 μM resulted in a > 50% reduction in active FAK and phosphorylated paxillin, which are overexpressed in osteosarcomas. These molecular changes were associated with significant inhibition of cell migration (mean of 50%) (27).

In vitro assays using isolated rabbit osteoclasts and bone slices have also shown that saracatinib (0.1-5.0 μM) concentration-dependently reduces areas of bone resorption, the number of resorption pits and the average area of the resorption pits, i.e., pathologies observed in osteoclast-driven metastatic bone disease and osteoporosis (28).

In vitro studies in a panel of 18 non-small cell lung cancer (NSCLC) cell lines have shown that saracatinib inhibits tumor growth in cell lines displaying wild-type and mutant EGFR (IC_{50} < 1 μM). Further analysis revealed an associated induction of G_1 arrest and apoptosis, inhibition of downstream signaling via STAT3 and ERK-1/2 and reducion of cell migration (29).

In vitro data have also been presented to suggest that saracatinib may be effective in acute lymphoblastic leukemia (ALL). Saracatinib concentration-dependently arrests cell growth and induces apoptosis (in 30-80% of cells) only in Philadelphia chromosome-positive (Ph^+) lymphoblasts at concentrations from 0.5 μM, without activity in Ph^- lymphoblastic cell lines. These effects are associated with inhibition of BCR/ABL autophosphorylation (which facilitates cellular transformation) (30).

The pharmacodynamic targets of saracatinib have been investigated in vivo in human colorectal, pancreatic, breast and lung tumor xenografts. Saracatinib-mediated antitumor activity was shown to be associated with reduced phosphorylation of the Src substrates FAK and paxillin (31). Immunohistochemical investigations of tumor samples from animals treated with saracatinib have confirmed a reduction in the Src substrates phosphorylated FAK and paxillin in the cell membrane and cytoplasm (32).

SCID mice injected with 2 x 10^5 PC-3 cells showed a delay in the formation of osteolytic lesions upon treatment with saracatinib (25 mg/kg/day), according to observations of populated osteoclasts in bone sections stained for hematoxylin and eosin and tartrate-resistant acid phosphatase (TRAP) (24).

In vivo investigations in rats bearing NIH/3T3 xenografts transfected with a constitutively active human c-Src kinase have shown that saracatinib provides significant tumor growth inhibition (> 90%) at doses of 6 and 10 mg/kg/day. Further studies using a single dose of 10 mg/kg [^{14}C]-labeled saracatinib confirmed extensive distribution to many tissues in these animals. Pharmacokinetic studies using a single dose of 25 mg/kg have also indicated approximately 40-fold elevated levels of saracatinib in tumor tissue versus plasma (33).

In a mouse model of invasive squamous cell carcinoma saracatinib proved effective against tumor promotion and malignant conversion. The agent provided a 26% reduction in the average number of papillomas per mouse when given at a dose of 10 mg/kg/day. Furthermore, initial carcinomas appeared in control animals at week 14 compared with week 23 in the saracatinib-treated animals. After 38 weeks, 68% of the control animals had a carcinoma compared with 35% of the saracatinib-treated animals (34).

Studies in SCID mice inoculated with TSU-Pr1-B1 human bladder carcinoma cells have shown that saracatinib (10 and 50 mg/kg/day)

significantly reduces tumor growth and the associated development of bone lesions for a period of 5 weeks (35).

Further studies in nude mice injected with NBT-II rat bladder carcinoma cells have shown that saracatinib (50 mg/kg/day p.o. over 2 months) delays tumor development and significantly inhibits lymph node metastasis even at lower doses (10 and 20 mg/kg). These effects correlated with attenuated cell migration and a marked decrease in paxillin phosphorylation (36).

PHARMACOKINETICS AND METABOLISM

Results from a study in 60 healthy volunteers administered multiple ascending doses of up to 250 mg once daily for up to 14 days showed a time to peak plasma concentrations (t_{max}) of approximately 6 h and a mean terminal elimination half-life ($t_{1/2}$) of approximately 40 h at steady state. Plasma C_{min} concentrations remained above the IC_{50} for Src kinase. These studies confirmed the potential for administering AZD-0530 as a once-daily oral dose (37).

SAFETY

A phase I single-ascending-dose study in healthy male volunteers (N = 27) receiving 2.5 and 1000 mg investigated the dose-limiting toxicity (DLT) of saracatinib. At low doses, toxicity was mild and included nausea and diarrhea; diarrhea and vomiting were dose-limiting at 1000 mg. It was determined that single doses up to 500 mg were well tolerated. A multiple-ascending-dose study in 60 volunteers randomized to saracatinib or placebo has shown that adverse events were generally mild and included rash, flu-like symptoms, myalgia, arthralgia, headache, loose stools and elevated creatinine at doses up to 250 mg (37).

A phase I study assessed the safety of saracatinib when administered in combination with cediranib (AZD-2171), a potent and selective inhibitor of vascular endothelial growth factor (VEGF) signaling, with activity against VEGFR-1, -2, and -3. Cediranib (20, 30 or 45 mg/day) for 7 days followed by daily treatment with cediranib at the same dose in combination with saracatinib 175 mg was well tolerated in patients with advanced solid tumors refractory to standard therapies (N = 18). No DLTs were reported during the first 28 days of treatment and common drug-related adverse events included diarrhea, hypertension and hoarseness. There did not appear to be a major effect of AZD-0530 on the steady-state pharmacokinetics of cediranib, and vice versa (38).

The clinical safety of saracatinib has also been investigated in patients with multiple types of cancers (N = 81) at daily doses of 50-250 mg. DLTs were recorded at 200 mg (n = 2; febrile neutropenia, dyspnea) and 250 mg (n = 3; leukopenia, septic shock [grade 5] with renal failure and asthenia). Daily doses of 50, 125 and 175 mg were well tolerated in this patient population (39).

A phase II study investigated saracatinib at 175 mg/day p.o. in metastatic colorectal cancer patients (N = 10) receiving 28-day treatment cycles. Toxicity profiling in these patients revealed only grade 3 toxicities not attributable to disease progression (hypophosphatemia [n = 6], hypocalcemia [n = 1], hyponatremia [n = 2], nausea [n = 1] and leukopenia [n = 1]), with 30% requiring a reduction in dose (40).

CLINICAL STUDIES

Data from randomized, double-blind, placebo-controlled, single- and multiple-ascending-dose studies in healthy male volunteers receiving 2.5-1000 mg saracatinib suggested inhibition of osteoclast-mediated bone resorption, as indicated by treatment-associated reductions in mean levels of biomarkers of bone resorption (serum *C*-terminal telopeptide of type I collagen [sCTX] and urine N-telopeptide corrected for urine creatinine [uNTX/Cr]) (41). Further investigations have also confirmed that saracatinib at 125 and 175 mg/day (given over 21 days) successfully reduces sCTX and uNTX/Cr (up to 60% reduction) in adult patients with advanced solid malignancies (42).

In the phase I study in patients with advanced solid tumors described above, stable disease had been reported in 100% of patients receiving cediranib 20 mg and saracatinib 175 mg (38).

Analysis of biopsy samples from patients in a phase I study confirmed saracatinib-mediated modulation of the phosphorylation of the Src substrates paxillin and FAK (39).

Median progression-free survival in the phase II study in patients with advanced colorectal cancer was reported to be 7.9 weeks (40).

Numerous clinical trials are under way evaluating saracatinib in a variety of cancers (43-60).

SOURCE

AstraZeneca (GB).

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