Involvement of PI3K/Akt Pathway in Prostate Cancer – Potential Strategies for Developing Targeted Therapies

Nicole Pommery and Jean-Pierre Hénichart*

Institut de Chimie Pharmaceutique Albert Lespagnol, Université de Lille 2, Lille, France

Abstract: This review presents some therapeutic interventions actually considered in prostate cancer therapy to compensate constitutive activation of the PI3K/Akt signalling pathway induced, particularly, by mutations of PTEN gene. Special emphasis is placed on applicability of EGF-R tyrosine kinase, COX-2, PDK-1, mTOR and farnesyltransferase inhibitors.

Keywords: Prostate cancer, PI3K/Akt/mTOR pathway, growth inhibition.

INTRODUCTION

Prostate cancer is the most invasive and frequently diagnosed malignancy and second leading cause of cancer death in men. Surgical or pharmacological castration remains the mainstay treatment for advanced and aggressive prostate cancer. This endocrine therapy induces dramatic growth arrest and cell death in sensitive prostate cells but mutations of androgen receptor (AR) and alterations in the relative expression of AR co-regulators contribute together with increased growth factor production, to the progression of prostate cancer [1].

Chemotherapy and radionuclides have a role in the palliative treatment of symptomatic metastatic disease [2]. However, there is currently no standard treatment for the growing group of patients with asymptomatic hormone-refractory prostate cancer even if numerous targets are evoked and evaluated in clinical trials [3-5].

Among genetic approaches more recently reported, only a few genes have been found to be aberrated in a significant proportion of prostate cancer [6]. With the cyclin-dependent kinase inhibitor $p27^{kip1}$, a target of Akt [7], PTEN (phosphatase and tensin homolog, deleted on chromosome 10) is one of the most commonly mutated tumour suppressor genes in this pathology [8,9]. Somatic deletions or mutations of this gene have been frequently identified and this may be playing a key role in tumour initiation and tumour metastasis in advanced cancers.

PTEN is the major 3-phosphatase in the phosphoinositol-3-kinase (PI3K)/Akt pro-apoptotic pathway and acts in direct antagonism to growth factor stimulated PI3-kinases. Early studies of PTEN showed that its activity was able to promote cell cycle arrest and apoptosis and inhibit cell motility [10] but more recent data have identified other functional consequence of PTEN action, such as effects on the regulation of angiogenesis [11-14]. So, in addition to activities on androgen receptor [15] or IGF-IR expression [16] via PI3K/Akt-independent pathways, PTEN regulates PI3K/Akt/mTOR pathway, which is now accepted as being at least as important as the Ras-MAP kinase pathway in cell survival and proliferation. PI3K activation leads to the generation of PIP3 in the membrane. PIP3 constitutes a second messenger for activation of downstream pathway including Akt and is tightly regulated by the opposing effects of PI3K and several PIP3 phosphatases among which PTEN is the most prominently involved in tumour genesis. The biologic effects of deregulation of Akt kinase activity are mediated by the downstream phosphorylation targets of Akt and by the impact of this phosphorylation on their functions.

The interaction of PIP3 with the pleckstrin homology domain of Akt recruits Akt to the plasma membrane where it is phosphorylated at two key regulatory sites, Thr^{308}, by 3-phosphoinositide-dependent protein kinase-1 (PDK-1) [17] and Ser^{473}, by an as-yet-unidentified kinase (PDK-2) [18]. Phosphorylation at both amino acids is necessary for full activation of Akt and the subsequent control of biological responses including apoptosis inhibition and cell cycle progression. Its several downstream effectors are implicated in increased survival (NF-kappaB, p53, caspases, Bcl-2 family members, forkhead transcription factors), stimulation of proliferation (cyclin-dependent kinase inhibitors $p21^{waf1}$ and $p27^{kip1}$, GSK-3), regulation of cell metabolism and cell growth (mTOR, PFK-2, $p70^{S6K}$) and angiogenesis (eNOS).

The development of strategies to specifically target PI3K/Akt is the subject of intense research efforts. This review will focus on some therapeutic interventions actually considered in prostate cancer therapy, particularly (Fig. **1**):

- inhibition of growth factor tyrosine kinase activities
- blockage of proteins which use Akt as effector(growth factor receptors, Ras, COX-2 ...) to avoid consequences of suractivation of this kinase
- new FRAP/mTOR inhibitors which already have demonstrated additive or synergistic antitumour activity when combined with conventional cytotoxic drugs
- inhibition of farnesyltransferase activity which could prevent, in addition to farnesylation of Ras, the activation of numerous steps of PI3K/Akt cascade such as Rheb, NF-kappaB, $p70^{S6K}$.

I – EGF-R Tyrosine Kinase Inhibitors

The development of therapeutic agents inhibiting the surexpression or abnormal activation of protein tyrosine

*Address correspondence to this author at the Institut de Chimie Pharmaceutique Albert Lespagnol, Université de Lille 2, Lille, France; Tel: +33-3-2096-4374; Fax: +33-3-2096-4906;
E-mail: henicha@pharma.univ-lille2.fr

1389-5575/05 $50.00+.00

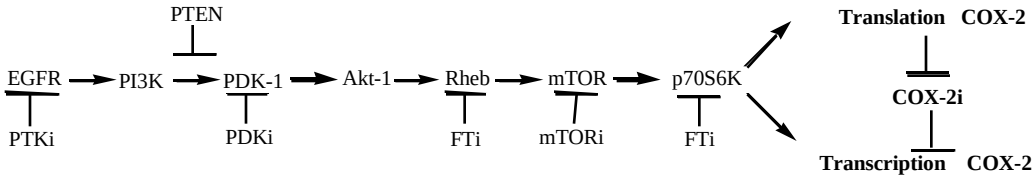


Fig. (1). Potential strategies for developing targeted therapies.

kinase is now a major field of research in both academic institution and pharmaceutical companies [19,20]. Because of their deregulation in prostate cancers and of experimental evidence of their role in the progression to hormone-refractory clinical behaviour [21], protein kinases in the epidermal growth factor-receptor (HER) family are among the protein kinases considered as prime targets for the development of selective inhibitors [22,23].

Several small molecule inhibitors have been developed [24,25] like erlotinib (OSI-774, Tarceva), CI-1033 (PD 183805), GW 2016, CP-654577 or PKI 166 and gefitinib (ZD 1839, Iressa) (Fig. **2**). ZD 1839, first described as a strong and selective inhibitor of erbB/EGFR tyrosine kinase [26] was found to act on the EGFR autophosphorylation and cellular proliferation of androgen-sensitive and androgen-independent human prostatic cancer cell lines and primary cultures derived from human prostatic cancer tissue [27]. This orally active anilinoquinazoline is currently under clinical evaluation in phase II to III clinical trials in patients with various tumour types [23], including prostate cancer trials ongoing as monotherapy, in combination with chemotherapy and/or radiotherapy, and other targeted agents [28].

A major disadvantage of this class of therapeutic agents is the rapid tumour regrowth after termination of therapy. However, their minimal adverse events justify further studies, which could explicit optimal conditions of ZD 1839 efficacy use in prostate cancer.

In vitro, ZD 1839 associated with a second generation hybrid oligonucleotide antisense MDM2 synergistically inhibits the growth of hormone-independent prostate cancer cells [29]. This effect is accompanied by the inhibition of MDM2, phosphorylated Akt, phosphorylated MAPK and vascular endothelial growth factor expression, supporting the clinical development of this therapeutic strategy.

Moreover, gefitinib is able to suppress proliferation and invasion of androgen-independent prostate cancer by

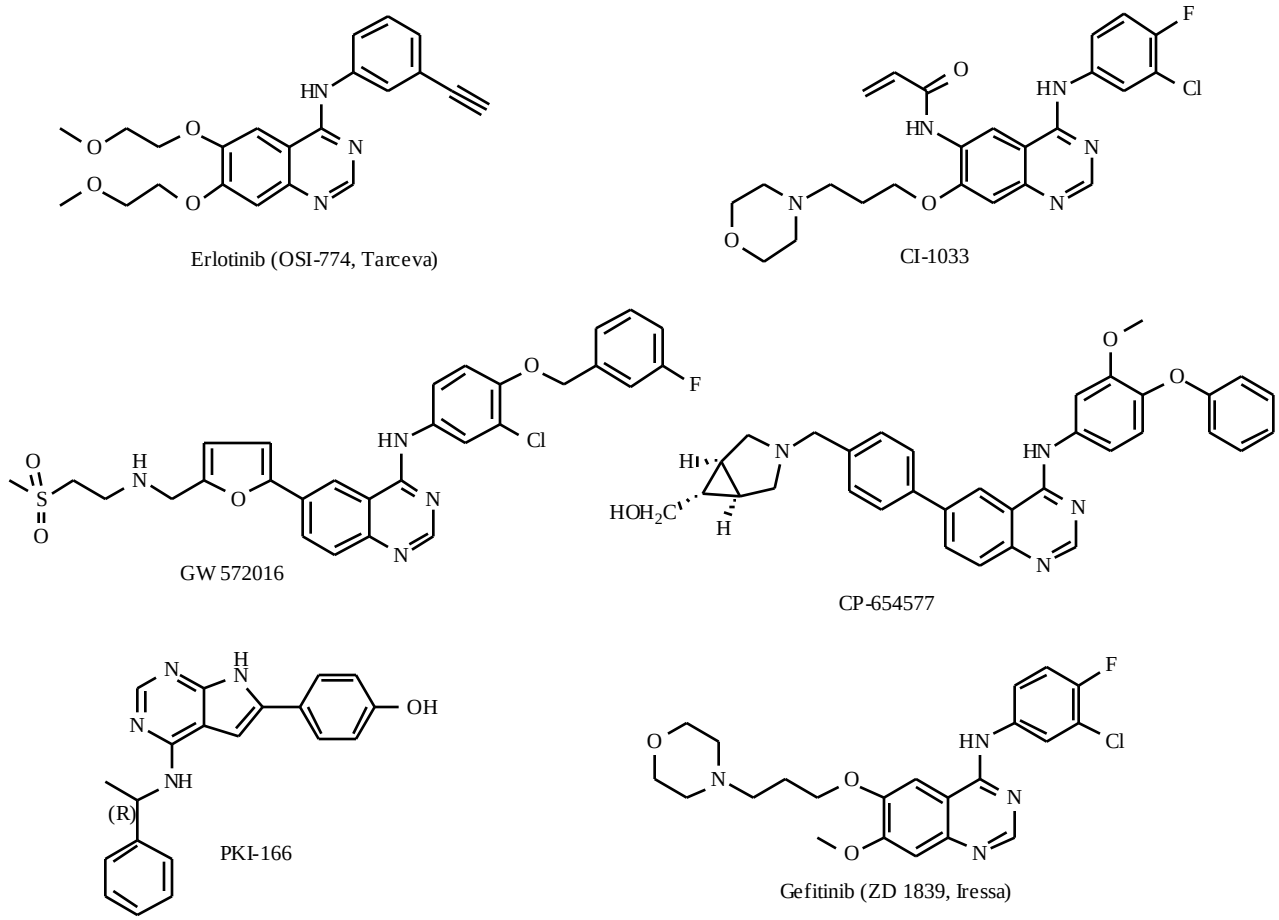


Fig. (2). Structures of developed tyrosine kinase inhibitors.

suppressing EGF-stimulated activation of the PI3K/Akt pathway [30]. Its antitumour effect require the subversion of Akt function but less of PTEN, in EGF receptor-expressing tumour cells, counteracts the antitumour action of EGFR tyrosine kinase inhibitors by permitting a high level of Akt activity independent of receptor tyrosine kinase inputs [31]. Thus, combined blockage of the EGFR tyrosine kinase and Akt should be considered as a therapeutic approach of prostate cancer.

The interaction between EGF-R and androgen receptor in prostate cancer cells [32,33] suggests another mechanism to explain that ZD 1839 is a possible therapeutic drug for patients with hormone-refractory prostate cancer. Several studies have suggested the involvement of growth factor receptor tyrosine kinases in ligand-independent activation of the androgen receptor and progression of prostate cancer and it was demonstrated that Iressa reduced the HER2/Neu-induced transactivation activity of the androgen-receptor in PC3 and DU 145 cells [34].

Convincing data of present studies stimulate intense research involving a number of small molecule inhibitors including the large aminoquinazoline family [35]. However, much more research needs to be done to clarify accurate mechanism [36] and to consider integration of this therapeutic strategy into the standard treatment of advanced cancer in the near future.

II – COX-2 Inhibitors and PDK-1 Inhibitors

Aspirin has been used to control pain and inflammation for over a century and, in the early 1980s, the chronic use of this drug and other non-steroidal anti-inflammatory drugs ($NSAID_s$) has been shown to reduce the risk of colon cancer. More recently, a similar protective effect has been demonstrated for $NSAID_s$ [37], and particularly, for the selective cyclooxygenase-2 (COX-2) inhibitor celecoxib (Fig. **3**), in multiple tissues including those of the breast, prostate and lung but the exact mechanisms that account for the anti-proliferative effects are still not fully understood and it is still controversial whether or not these effects are mediated through the inhibition of COX-2 activity and prostaglandin synthesis.

Studies on relationship of COX and prostate were initiated in 1993 [38] who analysed COX-1 and COX-2 mRNA expression in various human tissues and reported the highest levels in the prostate. Many other studies verified this observation and reported that compared to normal tissue, COX-2 and less consistently, the upstream and downstream enzymes of the prostaglandin synthesis pathway are overexpressed in human prostate cancer [39-42]. There is ample evidence that COX-2 and its PG products may play a critical role in prostate cancer development and progression. This is particularly based on numerous in vivo observations and on the assumption that prostaglandins and other COX-2-generated downstream mediators promote tumour cell proliferation, survival and angiogenesis [43, 44]. However, several authors indicated that a COX-2 independent mechanism may be involved in the antitumour effect of COX-2 inhibitors [45]. This is supported for example by the disparity by several orders of magnitude between the concentration needed to inhibit COX-2 and that for causing cell cycle arrest and apoptosis in vitro [46,47] or by the absence of correlation between the level of COX-2 expression and the drug activity.

First data demonstrated that selective COX-2 inhibitors induced apoptosis of human prostate cancer cell lines by down-regulating the anti-apoptotic protein Bcl-2 [48] but it is now accepted that the induction of apoptosis by celecoxib is independent of Bcl-2 and is mediated by interfering with multiple signalling targets, including Akt and ERK2 [49,50].

Kulp and collab. [51] demonstrated that a close celecoxib analogue deficient in COX-2 inhibitory activity, DMC (Fig. **3**), was able to inhibit PC3-cell proliferation in a manner qualitatively similar to celecoxib one, suggesting that COX-2 inhibition alone could not account for the *in vitro* antiproliferative effect of celecoxib. The authors suggested that Akt activity was attenuated as a result of the partial

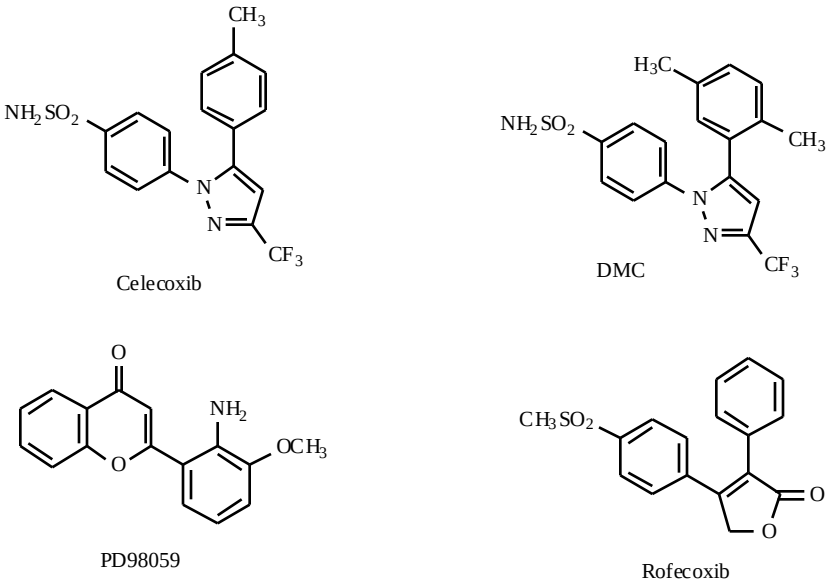


Fig. (3). Structures of Celecoxib and its analogue DMC, of PD98059 and Rofecoxib.

inhibition of PDK-1 in concert with the concomitant Akt dephosphorylation by protein phosphatase 2A. In addition, they reported that celecoxib (and DMC) facilitated the dephosphorylation of extracellular signal-regulated kinase 2 but this ERK 2 down-regulation might not be as important as PDK-1 inhibition in celecoxib-induced cell death, because treatment of PC3 cells with the inhibitor of mitogen-activated protein kinase kinase PD 98059 (Fig. **3**) did not lead to apoptotic death. Celecoxib should also be able to perturb intracellular calcium by inhibiting endoplasmic reticulum Ca^{2+}-ATPases [52].

Together, these data underscore the pharmacological complexity in COX-2 independent signalling mechanisms that celecoxib uses to mediate antiproliferative effects in vitro. The design of effective new apoptosis-inducing agents is the focus of many researches. The molecular structures of celecoxib and rofecoxib (two orders of magnitude lower than that displayed by celecoxib) (Fig. **3**) were used as starting points to determine structural requirements for the induction of apoptosis [53,54]. A bulky terminal phenyl ring, a heterocyclic system with negative electrostatic potential and a benzenesulfonamide or benzene carboxamide moiety seemed essential. The electronic nature of the central ring system seems to be important element to the antiproliferative activity [55]. Considering PDK-1 as a target, it was observed that replacement of the sulfonamide function with 2-aminoacetamide and guanidine led to improved PDK-1 inhibition [56].

PDK-1 is an activator of PKB, which plays a key role in the phosphorylation of downstream effectors such as NF-κB, mTOR. So, PDK-1 is a highly attractive target for therapeutic intervention.

Crystal structures of several bisindolyl-maleimide-based PKC inhibitors, such as LY-333,531 and UCN-01, in complex with PDK-1 have been determined [57-59]. The most significant finding of these studies is the unusual way in which these inhibitors bind PDK-1 catalytic active site : the 7-hydroxy group of UCN-01 which is not present in the structure of the parent drug PKC inhibitor staurosporine (Fig. **4**), generates hydrogen bonds with active-site residues such as Thr^{222} and Gln^{220} via an ordered water molecule. Moreover, it has recently been noted that the pleckstrin homology domain of PDK-1 may also provide a druggable target [58]. The recruitment of PKB and its PDK-1 activation is an essential event in triggering the PI3K signalling cascade.

In the light of the prominent role of PDK-1/Akt signalling in different stages of tumourigenesis, development of this new class of anti-tumour agents via optimisation of celecoxib-based on one hand and bisindolyl-maleimides-PKC inhibitors-based on the other hand, is particularly hopeful for the prevention and/or therapy of cancers alone or in combination with other treatments [60].

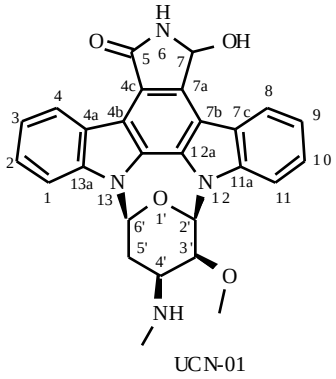
III – mTOR Inhibitors

m-TOR (mammalian target of rapamycin), also known as FRAP (FK506-binding protein 12 – and rapamycin – associated protein), RAFT 1 (rapamycin and FKBP-12 target-1) or RAPT 1 (rapamycin target-1) is a 289 kDa serine/threonine kinase highly conserved from yeast to mammals. TOR kinase activity is regulated by nutrients and growth factors, through both PI3K and Akt/PKB pathways.

Two translational components ribosomal S6K1 and eIF4E binding proteins (4E-BPs), are the best-characterized downstream effectors of mTOR. In addition, mTOR is involved in the regulation of cyclins D1/A, cyclin-dependent kinases, cyclin-dependent kinase inhibitors ($p21^{Cip1}$ and $p27^{Kip1}$), retinoblastoma protein, RNA polymerase I/II/III-transcription and translation of rRNA and tRNA, protein phosphatases (PPA2, PP4 and PP6), hypoxia – inducible factor-1α (HIF-1α), vascular endothelial growth factor and CLIP-70. So, this protein kinase is an important and crucial actor in PI3-kinase dependent oncogenesis, with a central role in the regulation of cell proliferation, growth, differentiation, migration and survival [61,62].

Suppression of mTOR and the survival signals that target mTOR offer an opportunity to reactivate default apoptotic pathways in cancer cells [63]. Concerning prostate cancer, although PI3K signalling has been hypothesized for few years to play an important role in human prostate cancer cells [64-66], a better understanding of the molecular mechanism for prostate carcinoma development is still necessary.

Reported effects of PI3K can be mimed by mTOR inhibition. Such is the case for inhibitory effects on G1 cell cycle progression and expression of cyclin D1, CDK4 and Rb phosphorylation [67]. Furthermore it was reported that mTOR inhibition induced apoptosis of prostate epithelial cells. Induction of cell death required the mitochondrial pathway. However, Bcl2 expression only partially restored cell growth in the setting of mTOR inhibition. Several



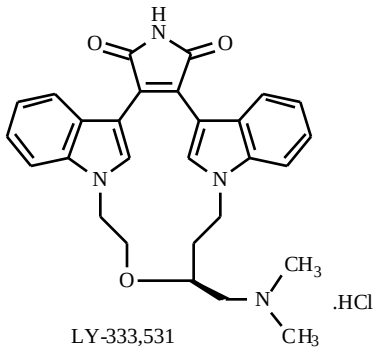


Fig. (4). Structures of UCN-01 and LY-333,531.

authors showed that hypoxia-inducible factor 1 (HIF-1)α, the regulated subunit of the transcription factor HIF-1 activates the transcription of a number of genes that mediate angiogenesis and tumour formation and was a major m-TOR-dependent survival signal [68-71]. Stimulation of HIF-1α protein expression stimulates the expression of VEGF and then tumour angiogenesis [72]. These data demonstrate that pharmacological agents that target mTOR in tumour cells may increase the therapeutic efficacy in prostate cancer therapy.

Three potent and specific mTOR inhibitors have been reported: rapamycin, CCI-779 (cell-cycle inhibitor-779; Wyeth-Ayerst, PA, USA), and RAD001 (also called everolimus; Novartis AG, Basel, Switzerland) (Fig. **5**) [73].

Rapamycin, a macrocyclic lactone was originally identified as a fungicide isolated from the soil bacterium Streptomyces hygroscopicus. The three rapamycin analogues are more suitable for clinical use than rapamycin because of their improved pharmaceutical properties (water-solubility and stability in solution) without any change in cellular effects and are undergoing clinical trials.

Rapamycin inhibits the FK506-binding protein 12 (FKBP12) / mammalian target of rapamycin (mTOR) complex and causes cell cycle arrest in G1 [74,75]. Rapamycin initially forms a complex with cyclophilin FKBP-12. This complex then binds to the FRB domain of mTOR and inhibits the function of mTOR. It seems that rapamycin disrupts substrate recognition instead of directly inhibiting phosphotransferase activity [76]. The FKBP-12 / rapamycin complex bound to mTOR destabilizes the mTOR-zaptor-4E-BP1/56K1 scaffold complex, leading to dephosphorylation of S6K1 and 4E-BP1 and then inhibits both synthesis of specific proteins and G1 cell-cycle progression [62].

However, genetic mutations or compensatory changes in tumour cells influence the sensitivity of rapamycins [77]. These mutations or defects first of mTOR and FKBP-12 or second of mTOR-regulated proteins (S6K1-4E-BP1-PP2A-related phosphatases and $p27^{KIP1}$) confer rapamycin resistance.

On the other hand, reduced PTEN expression, as in prostate cancer cells, and related to poor prognosis and resistance to chemotherapy and hormone therapy, confers susceptibility to rapamycins because, under conditions of PTEN deficiency, the PI3K/Akt signalling pathway becomes a fundamental proliferative and survival pathway [78].

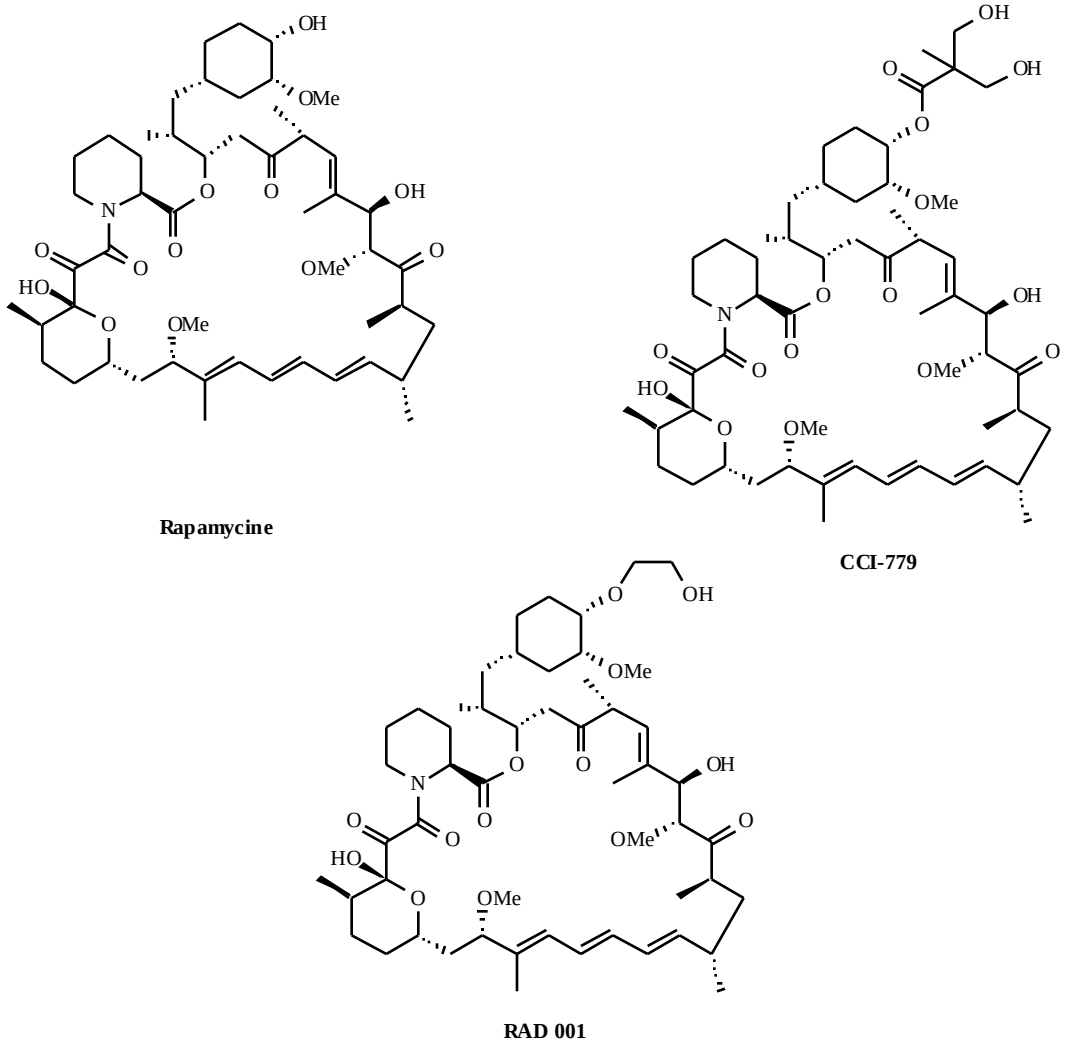


Fig. (5). Structures of three specific mTOR inhibitors.

Preliminary efficacy data from the single agent studies indicate that these agents are more likely able to induce growth inhibition rather than tumour regression [79]. Combinations with cytotoxic chemotherapy, particularly when PTEN mutation confers resistance against such chemotherapy by bcl-2 expression and inhibition of apoptosis, have been evoked. No clinical data were available but it is demonstrated that inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells [80].

In addition to design new members in this class of antitumour drugs, developmental challenges include now the ability to predict which tumours will be particularly sensitive to mTOR inhibitors.

IV – Farnesyltransferase Inhibitors

Over the last several years, protein prenylation has been the subject of intense study and has been found to be critical for the function of key proteins involved in signal transduction [81,82]. Prenylation is a form of lipid modification in which either a C-15 farnesyl or C-20 geranylgeranyl group is covalently attached via a thio ether linkage to the cysteine residue of proteins near the carboxy terminus. These proteins belong to a group termed "CAAX proteins". Cysteine is modified by the protein farnesyltransferase (PFTase) when X is methionine, serine, glutamine or cysteine, and by protein geranylgeranyltransferase when X is leucine or phenylalanine.

Due to the functional role of Ras farnesylation, farnesyltransferase inhibition was first thought to be a strategy for interfering with Ras-dependent transformation. When farnesylation is blocked, the function of Ras protein [83,84] is severely impaired because of the inability of the non-farnesylated protein to anchor to the membrane.

Several strategies have been developed to inhibit the farnesylation of Ras, the most common one being the design of compounds that mimic the carboxy-terminal CAAX motif of Ras and compete for binding to farnesyltransferase. For example, the peptide containing the amino acid sequence Cys-Val-Phe-Met (CVFM) is a potent inhibitor of PFTase and is competitive with Ras binding with a Ki of 60 nM. Through a massive effort by many leading pharmaceutical companies, a large number of highly effective farnesyltransferase inhibitors (FTIs) have been identified and developed as potential cancer therapies [85,86]. However, later works suggested that farnesyltransferase inhibitors suppress cancer cell proliferation through mechanisms other than inhibiting Ras isoprenylation. Investigations have revealed a crucial role for alteration of the Rho family protein RhoB in the FTI mechanism [87,88]. Nevertheless, the fact that RhoB is farnesylated and geranylgeranylated wakes its candidature as a target for FTI somewhat complex and several key observations argued against the involvement of RhoB in mediating FTI antitumour activity [89].

Another hypothesis is that FTIs induce accumulation of human cancer cells in the G2/M phase of the cell cycle due to inhibition of the prophase/metaphase transition during mitosis. After exposure to FTIs, cells are unable to form bipolar spindles and their chromosomes failed to align to form a metaphase plate [90]. Centromere-associated proteins (CENP-E and CENP-F) were investigated but further studies are needed in order to elucidate whether or not these proteins may be involved in the mechanism by which FTIs inhibit cell cycle progression [91]. Finally, another reported candidate FTI target is Rheb, a farnesylated Ras-related protein activator of the mTOR/p70S6K signalling pathway [92]. This could confirm that a mechanism for FTI inhibition of human tumour growth is by inducing apoptosis through inhibition of PI3K/Akt-2-mediated cell survival and adhesion pathway [93].

Although FTIs have been shown to antagonize oncogenic signalling, reverse malignant transformation, inhibit human tumour growth and induce tumour regression without any signs of toxicity, their mechanism of action is not known. However, many investigators have developed farnesyltransferase inhibitors as novel anticancer drugs [94-97]. Three FTIs (Fig. **6**) are undergoing clinical testing currently as monotherapy and in combination with standard cytotoxic agents:

R115777 (Zarnestra^{TR}) is a non-peptidomimetic quinolone analogue and seems promising in pancreatic and colorectal cancers [98].

SCH66336 is a tricyclic, non-peptidomimetic specific reversible inhibitor of FT. It has activity in a wide variety of human tumour xenografts including prostate and could have synergistic cytotoxicity with taxanes [99].

BMS-214662 is a benzodiazepine-type FTI. Data from two phase I combination trials with weekly BMS-214662 infusion and paclitaxel or cisplatin chemotherapy have been

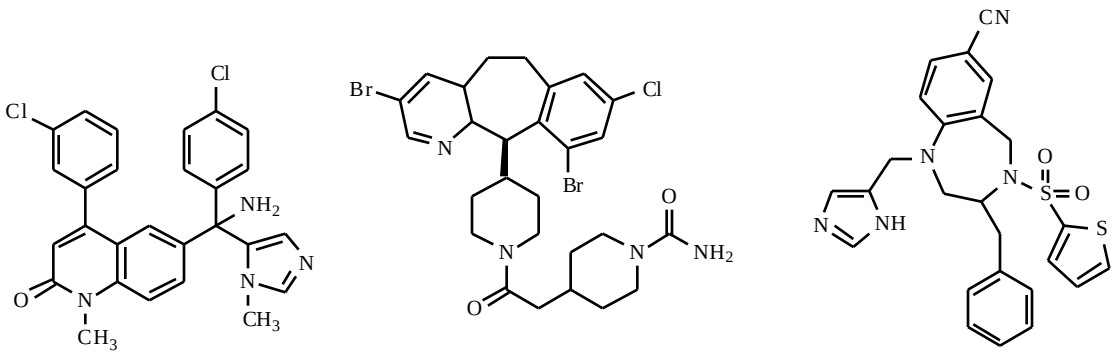


Fig. (6). Structures of three farnesyltransferase inhibitors undergoing clinical testing.

reported in patients with prostate cancer [95] but there are currently no published phase II trials with this agent. No real clinical data of the use of FTIs in prostate cancer are available but further investigation could be performed to test interest of this class of antitumour drugs, which targets PI3K/Akt pathway dramatically activated in this pathology. Indeed, although clinical experience with FTIs is at once striking and disappointing, ways of enhancing the possibility of a successful FT inhibitor anticancer drug are actively discussed [100]. At present, as monotherapy, FTIs appear to produce significant efficacy in myeloid leukaemias [101] and breast cancer [102] and, furthermore, like other cancer therapeutics, FTIs will probably be more effective when used in combination and particularly with taxanes [103], chemotherapy particularly useful in prostate cancer [104].

CONCLUSION

In prostate cancer, growth factor receptors are overexpressed and in monotherapy or in combination, promising agents target erbB2/HER1/EGFR (Iressa) or PDGF receptor (Glivec). But a drug-resistance could appear due to the activation of the survival Akt pathway.

The PI3K/Akt signalling was found to be indispensable for tumour cells but not necessarily for normal tissues and thus, giving a new hope for the design of selective anti-cancer drugs acting on one or several steps of this important pathway. Moreover, it has been observed that certain hormone-independent prostate cancer cells could prolifer as a consequence of a deletion of the PTEN gene.

Thus, the most effective use of mTOR inhibitors, for example, in combination with inhibitors of tyrosine kinases, with inhibitors of PDK-1 and with inhibitors of enzymatic activation, such as farnesyltransferase inhibitors, could be a very promising approach for therapeutic intervention in the prostate cancer.

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