1 INTRODUCTION

1.1 Global cancer statistics

The global burden of cancer continues to increase largely because of aging and growth of the world population alongside increasing adoption of cancer-causing behavior, particularly smoking, in economically developing countries. GLOBOCAN 2008 estimated about 12.7 million cancer cases and 7.6 million cancer deaths in 2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. Lung cancer is the leading cancer site in males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths. Further, the mortality burden for lung cancer among females in developing countries is as high as the burden for cervical cancer, each accounting for 11% of the total female cancer deaths. Although overall cancer incidence rates in the developing world are half of those seen in the developed world in both the sexes, the overall cancer mortality rates are generally similar. Cancer survival is too low in developing countries, most likely because of late stage at diagnosis and limited access to timely and standard treatment.[1,2]

The diagnosis of cancer carries great physical and mental suffering for the affected individuals and poses a significant burden on the health care system. For many tumors, conventional management strategies (surgery, radiation and chemotherapy) have high toxicity with marginal efficacy. The consensus that has emerged among investigators is that surmounting the cancer therapeutic problem can be greatly facilitated by an in depth understanding of the molecular genetics underlying individual malignancies.[3] In most tissues, the proliferating neoplastic cells remain in contact with one another, eventually forming a tumor. A portion of cells, however lack adhesiveness and become easily detached from the main mass causing metastases.

Tumors can broadly be classified into two categories, i.e. benign and malignant tumors. A benign tumor is localized, slow growing, well differentiated and generally harmless throughout the life span of the organism. Malignant tumors, on the other hand, invariably endanger the life of the organism; the growth may obliterate the organ in which it

arises, may impair the function of adjoining organs by sheer pressure or most serious of all may shed cells which takes roots as metastases elsewhere in the organism. Malignant growth and metastases vary in the extent to which it retains the structure and the functional capacity of the tissue from which it originated; but frequently there is anaplasia or loss of characteristic tissue structure (dedifferentiation).

A malignant tumor is termed carcinoma if it arises in epithelial tissues such as skin or breast and sarcoma if it arises from non-haemopoietic mesenchymal tissues e.g. connective, vascular, maningeal and muscular.

1.2 Chemotherapy and its limitations

Unfortunately, most patients have tumors that are either incurable at diagnosis or are likely to relapse. Although 5-year survival has improved somewhat over the past three decades[4] and chemotherapy has an established role in the treatment of locally advanced and metastatic conditions, it is apparent that the pace of progress in the direction of cancer cure has remained too slow. Furthermore, recent studies suggest that the benefits attained with conventional cytotoxic combination regimens may have reached a plateau.[5]

There are currently over 90 anticancer drugs approved by the FDA. A majority of these are indiscriminate poisons of the cell replication machinery and several of them are 40-50 years old. However, a relatively recent study by the WHO concluded that curable cancers and those cancers where the cost benefit ratio clearly favors drug treatment can be managed with regimens based on only 17 drugs. In addition, surgery still remains the main anticancer modality accounting for at least 80 % of cancer “cures” while cancer chemotherapy is effective only in 10 % of all cancers. Deaths from cancer are projected to continue rising worldwide, with an estimated 12 million cases in 2030. (World health report 2008). Therefore, the need for new, efficacious cancer therapy is clearly evident.

What has changed in recent years? Cancer research has generated a rich and complex body of knowledge showing that cancer cells acquire numerous features that differentiate them from their normal counterparts. These functional differences arise from the acquisition of multiple genetic changes affecting a variety of cellular pathways. The mutation and deregulation of cancer genes lead to a wide range of changes in cellular structure and functions, all of which contribute to the malignant phenotype and pathological behavior of human cancer. Hanahan and Weinber have usefully characterized cancer in terms of six

hallmark traits: (1) self-sufficiency in growth signals; (2) insensitivity to growth inhibitory signals; (3) evasion of apoptosis; (4) limitless replicative potential; (5) induction of sustained angiogenesis; and (6) invasion and metastasis.[6] Laboratory experiments have demonstrated that, several of these essential alterations are necessary for the direct tumorigenic transformation of normal human epithelial and fibroblast cells.[7] Recent advances in biochemistry and pharmacology along with a more thorough understanding of cancer biology have led to the development of novel agents that selectively target malignant cells and their supporting elements. New agents, specifically designed to target important molecules in the malignant process, have been shown to cause tumor regression, and in some cases, prolong survival in cancer patients e.g. imatinib (Gleevec) targets the characteristic BCR-ABL anomaly in chronic myelogenous leukemia (CML) and C-KIT in gastrointestinal stromal tumors (GIST), trastuzumab (Herceptin) targets HER-2 in breast cancer, rituximab (Rituxan) targets CD20 in lymphomas, and bortezomib (Velcade) targets the proteasome in multiple myeloma. These agents are viewed as more cancer selective and are generally associated with reduced toxicity compared to traditional cytotoxics.[8] The need for targeted therapy in cancer has been apparent.

1.3 Role of tyrosine kinases in cancer

Protein kinases are enzymes catalyzing the transfer of the terminal (γ) phosphoryl group of ATP to specific amino acid residue, thus altering protein structure and ultimately affecting ligand binding and catalytic activity. Based on the specificity of the catalytic activity, protein kinases can be subdivided into tyrosine, serine and threonine kinases. The protein kinase compliment of the human genome (kinome) consists of 30 tyrosine kinase families containing about 90 distinct protein tyrosine kinases (PTKs), of which 58 members are receptor tyrosine kinases (RTKs).[9]

In 1980s Cohen isolated the epidermal growth factor (EGF) and the EGFR; the first receptor tyrosine kinase was identified 12 years later by a research team led by the same investigator.[10-12] He discovered that certain carcinogenic viruses (Erythroblastosis tumor viruses) contained the genetic information for the production of an altered human epithelial growth factor receptor (EGFR1), such growth factor receptors are found on the surface of cells and normally play a role in the regulation of cellular growth and differentiation process. The discovery that these receptors are present in unusually high numbers in many tumors, suggested that they played a role in carcinogenicity.

Since early 2003, Herceptin (Trastuzumab) for the treatment of advanced breast cancer, Gleevec (Imatinib mesylate, STI-571) for the therapy of patients with Philadelphia chromosome-positive chronic myelogenous leukemia and GIST (gastrointestinal stromal tumors), and Iressa (Gefitinib, ZD1839, in Japan) for the treatment of lung cancer have been the leading examples of drugs which have successfully translated basic research on oncogenes into cancer therapeutics.[13] These agents provide clinical validation of the emerging field of molecular oncology, specifically therapies targeting deregulated cellular pathways that play a critical role in tumorigenesis.[14-16] Very interestingly, these three drugs are inhibitors of protein tyrosine kinases, a class of enzymes which have been at the early onset of the discovery of oncogenes. Furthermore, they inhibit a subclass of these oncogenes, the receptor tyrosine kinases (RTKs), which present distinctive advantages as potential drug targets.

The activating mutations and/or overexpression of RTKs and their ligands can drive deregulated cell growth; RTKs such as the insulin-like growth factor 1 (IGF1) receptor are key components of the cell death control machinery; and last but not the least, angiogenesis-initiating signaling involves RTKs such as the vascular endothelium growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) receptors. As all of these traits can be targeted by the new generation of molecular therapeutic agents, this should place RTKs at a very prominent place among targets to attack the malignant diseases. Indeed, Sir Philip Cohen has very recently proposed that protein kinases in general may prove to be the major drug targets for the 21st century [17] in cancer and other diseases.

1.3.1 Epidermal growth factor receptors (EGFRs)

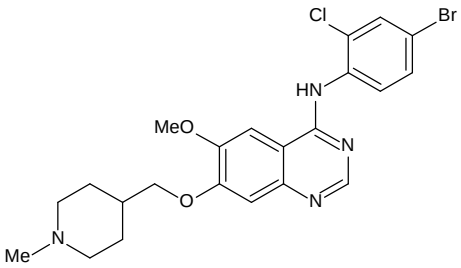
This is the most important category of receptors involved in the genesis of cancer. A detailed account of these receptors and the agonists and antagonists is given in section 1.4

1.3.2 Vascular endothelial growth factor receptors (VEGFRs)

The formation of new blood vessels from pre-existing ones, termed angiogenesis, requires the integration of highly complex and coordinated processes and is crucial for the expansion and progression to malignancy of solid tumors.[18] VEGFs bind to two high-affinity RTKs, VEGFR-1 and VEGFR-2 on endothelial cells.[19] VEGFs and VEGFRs are expressed at high levels in many types of human solid tumors, including glioma, lung, breast, renal,

ovarian and gastrointestinal tract carcinomas. Bevacizumab (RhuMab VEGF; Avastin) is a recombinant humanized monoclonal antibody which in combination with a 5-Fluorouracil/Leucovorin/Irinotecan-based chemotherapy increases response rates, time to disease progression and patient survival and has been recently approved as a first-line treatment for metastatic colorectal cancer.[20]

Small-molecule inhibitors target the ATP-binding pocket of VEGFRs. The first generation of these compounds includes indoline derivatives, the phthalazine derivative PTK787 (Vatalanib) and ZD6474 **(1)**, that is based upon a quinazoline template. ZD6474 potently inhibits several RTKs and is under phase III clinical trials for a variety of tumors.[21]



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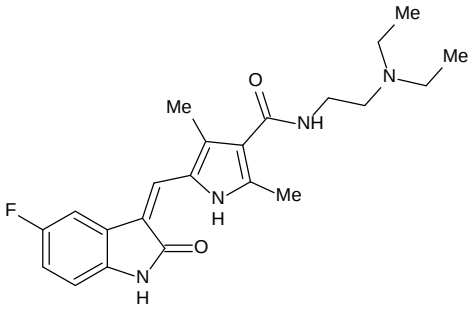
The second-generation kinase inhibitors' development was largely based upon molecular modeling targeting the active ATP-binding pocket. AAL993 is a molecule designed upon the PTK787 structure. It displays good biopharmaceutical properties and an excellent oral bioavailability in animal models. Clinical trials have not yet been initiated.[22]

1.3.3 Platelet-derived growth factor receptors (PDGFRs)

PDGFRs contain one α and one β chains that can homo- or hetero-dimerize upon binding to five different PDGF isoforms.[23,24] PDGFR signaling is important for the autocrine stimulation of cancer cells, in the paracrine stimulation of stromal fibroblasts and of perivascular cells, and in angiogenesis.[25]



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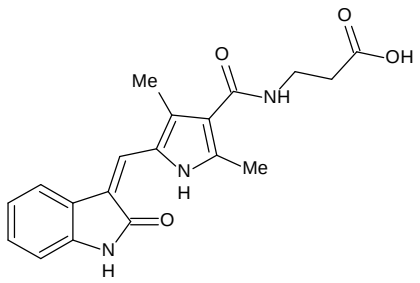

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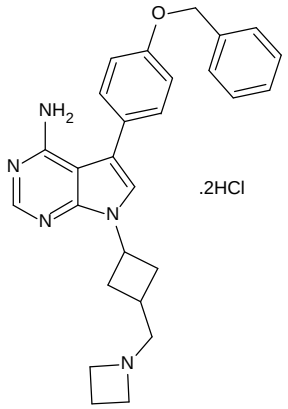
The most important inhibitor of PDGFRs is STI571 (Gleevec, Glivec, Imatinib), which is also used to inhibit the oncogenic activity of Kit and Abl in GIST and chronic myelogenous leukemia, respectively.[26] SU11248 **(2)** and SU6668 **(3)** are broad spectrum RTK inhibitors that potently enhance the regression of different PDGFR-expressing tumor types in preclinical studies, particularly in combination with chemo- or radiotherapy.[27]

1.3.4 Insulin-like growth factor I receptor (IGF-IR)

IGF-IR is an ubiquitously expressed RTK consisting of two extracellular α subunits, which bind the IGF-I and IGF-II ligands, and of two cytoplasmic β subunits that contain the kinase domain. IGF-IR is responsible for the development of several malignancies, like carcinomas of breast, prostate, liver, colon, pancreas and lung, and many compounds express their tumorigenic potential only in the presence of IGF-IR.[28] Several strategies for the inactivation of IGF-IR have been developed. Blocking antibodies or single-chain humanized scFv-Fc immunoconjugates raised against the α subunit of the receptor down regulate IGF-IR, reduce the survival of cancer cells and cause regression of pancreatic tumor xenografts in mice. The low-molecular weight kinase inhibitor NVP-ADW742 inhibits the growth of small cell lung cancer cells, [29] whereas NVP-AEW541 **(4)** reduces the growth of IGF-IR-driven fibrosarcomas in mouse xenografts.[30]



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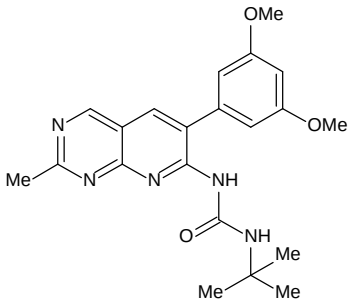
1.3.5 Fibroblast growth factor receptors (FGFRs)

The family FGFRs contains four receptor types that bind to 23 different ligands, the fibroblast growth factors (FGFs), whose prototypic members are acidic FGF (aFGF or FGF-1) and basic FGF (bFGF or FGF-2). FGFs and/or FGFRs are aberrantly expressed in several malignancies, and a high FGF serum level correlates with poor prognosis and resistance to chemotherapeutics.[31]

Development of pyrido-pyrimidine derivatives led to the development of several agents (PD166285, PD166866 **(5)**, PD173074 and PD161570) that selectively inhibit FGFR autophosphorylation and signaling in various cell models.[32,33] Association of a broad-spectrum (PD166285) and of a selective (PD173074) kinase inhibitor of this family in combination with photodynamic therapy causes tumor regression in animal models. The quinazoline derivatives ZD4190 and ZD6474 abrogate the proliferation of endothelial cell, and are currently under preclinical evaluation.[34] The oxindole derivatives SU6668, SU5416 and SU5402 inhibit vascularization and growth of tumor xenografts of diverse origins. Currently SU6668, also used in combination with chemotherapeutics, is undergoing phase II clinical trials.



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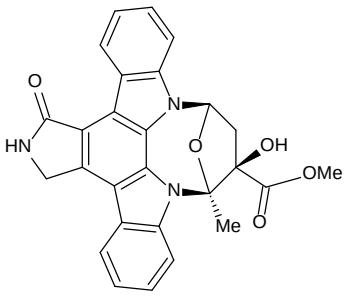
1.3.6 Hepatocyte growth factor receptor (HGFR)

The HGFR, encoded by the proto-oncogene *MET*, is functionally deregulated in a variety of cancer types. MET overexpression strictly correlates with higher metastatic potential and poor prognosis in a plethora of aggressive tumors, including thyroid and colorectal carcinomas, whereas its tyrosine kinase activity is increased by germ-line and somatic mutations in papillary, renal, gastric and hepatocellular carcinomas, and in lymph-node metastases of head and neck squamous-cell carcinomas.



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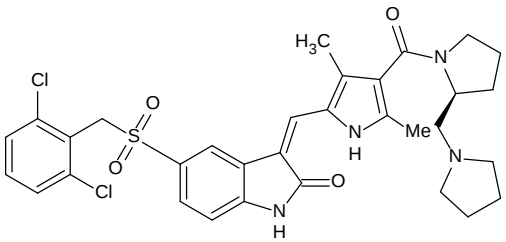

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The furanosylated indolocarbazole K252a **(6)** and the pyrrole-indolinone PHA-665752 **(7)** competitively inhibit the binding of ATP to the MET catalytic domain. Both compounds abrogate the activation of Met pathway and prevent the *in vitro* oncogenic

properties driven by c-MET.[35] The indoline derivative SU11274 is a highly selective MET inhibitor that induces G1 cell cycle arrest and apoptosis by targeting key regulators of the PI3K pathway.[36]

1.4 Endothelial Growth Factor Receptors (EGFRs) and related issues

1.4.1 Introduction

In the early 1980s, Mendelsohn and Baselga hypothesized that if binding of EGF to its receptor could be blocked, receptor activation and cell proliferation might be abolished. Several anti-EGFR monoclonal antibodies were developed with inhibitory activity against cells bearing EGFR. The inhibitory effects of these monoclonal antibodies in cell culture experiments were confirmed *in vivo* against human tumor xenografts expressing EGFR. Other investigators pursued development of compounds that inhibited the EGFR tyrosine kinase. Results with these inhibitors confirmed and extended the previous observations. These experiments established EGFR blockade as a promising approach for targeting cancer therapy.[37] Involvement of various tumors is listed in **Table-1**.

Table 1 EGFRs expression in solid tumours[38]

Tumour type (Range of tumours)	Expressing EGFRs (%)
Head and neck	80–100
Colorectal	25–77
Pancreatic	30–50
Lung	40–80
Esophageal	71–88
Renal cell	50–90
Prostate	40–80
Bladder	53–72
Cervical	54–74
Ovarian	35–70
Breast	14–91
Glioblastoma	40–50

1.4.2 Structure and types of EGFRs

The EGFRs or human epidermal receptors (HERs) form a family of four related RTKs broadly expressed in epithelial and mesenchimal tissues: EGFR (HER1, ErbB1), HER2 (neu, ErbB2), HER3 (ErbB3) and HER4 (ErbB4).[39] The complete primary structures of the ErbB family members were elucidated by molecular cloning.[40-45] Subsequent X-ray crystallographic studies of the extracellular and intracellular domains (ECDs and ICDs, respectively) of ErbB receptors alone or interacting with EGF and other ligands , or of ErbB receptor ECDs interacting with therapeutic MAbs, have further connected the structures with functions. Though variations exist in terms of ligand binding and protein tyrosine kinase (PTK) activity, the overall domain structure of the EGFR is archetypal for all four ErbB receptors (**Fig. 1**). All are large, modular proteins that are glycosylated on their ECDs and have molecular masses of ~180 kDa as determined by SDS-polyacrylamide gel electrophoresis.[46-53]

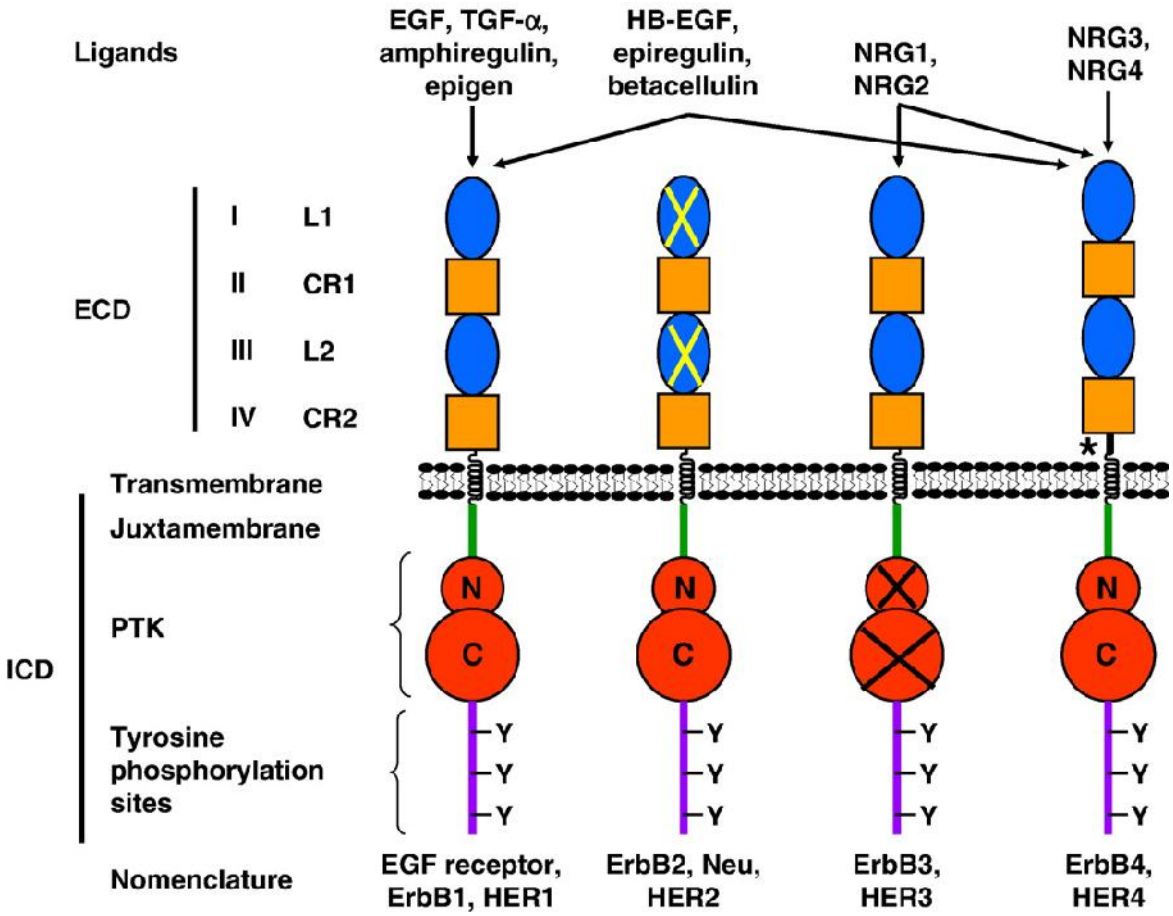


Fig. 1 *ErbB receptors and their ligands.*

1.4.2.1 Primary and domain structures of the EGFRs

The human EGFR is synthesized as a 1210-residue proreceptor (molecular mass of the polypeptide backbone: 134 kDa) that includes a post-translationally-cleaved 24-residue N-terminal signal peptide (including the N-terminal initiator Met−) to direct the pro-receptor to its correct cellular location in the plasma membrane. The mature 1186-residue protein **(Fig. 1**) consists of the N-terminal ECD (residues 1–621), a single transmembrane domain (residues 622–644), and an ICD consisting of a juxtamembrane sub-domain (residues 645–684), an intracellular PTK sub-domain (residues 685–953) and a non-catalytic C terminal regulatory region (residues 954–1186). The glycosylated ECD is made up of four smaller sub-domains of which two (sub-domains I and III, alternatively called L1 and L2, respectively) are homologous Leu-rich domains consisting of β-helix folds and the remaining two are Cys-rich (sub-domains II and IV, or CR1 and CR2) which are each internally crosslinked by disulphide bonds. The short transmembrane domain is typically rich in hydrophobic amino acids and is flanked on the intracellular side by a juxtamembrane region that has a regulatory role in receptor downregulation. The catalytic PTK sub-domain is a bilobed structure predicted to bind ATP in the region between the two lobes initially on the basis of homology with other PTKs.

The PTK sub-domain is unusual because, unlike other RPTKs, it does not require phosphorylation of a conserved Tyr-residue (Tyr845) in its activation loop (residues 831–852) to promote PTK activity. The smaller amino-terminal lobe (N-lobe) consists of residues 685–769 and forms an antiparallel β-sheet structure that also contains one α-helical region (the αC-helix), whereas the larger carboxyl-terminal lobe (C-lobe, residues 773–953) is predominantly α-helical. The C-terminal region contains several Tyr-residues which become phosphorylated on receptor activation and this is important in downstream effector binding.[54,5]

1.4.2.2 ErbB2

ErbB2 is similar in overall structure to the EGFR, but it is not a receptor in the accepted sense in that it has no known extracellular ligand (**Fig. 1**). However, it does possess a catalytically-active PTK sub-domain. Rather, it is a co-receptor which heterodimerises with other activated ErbB family members in spite of being unable to bind to ligands itself. The ECD normally assumes a conformation similar to that of the “activated” EGFR, i.e. the EGF-EGFR complex, [47,50] though the ErbB2 PTK is enzymatically inactive in its monomeric form.

Some of the residues critically involved in the intramolecular tether between sub-domains II and IV in the inactive EGFR are absent in ErbB2 and, consequently, the dimerisation arm on sub-domain II is exposed and available. In addition, residues that play a role in ligand binding in both the EGFR and ErbB3 are replaced with residues which hinder ligand binding.[54,55] ErbB2 is the preferred heterodimerisation partner of all other ErbB receptors and increases their ligand binding affinity.[56] Thus, ErbB2 decreases the rate of EGF dissociation from the EGFR, resulting in prolonged activation of EGFR signalling pathways. Moreover, heterodimers containing ErbB2 are more stable and are endocytosed at a lower rate than other heterodimeric ErbB combinations.[57–61] Unlike EGFR homodimers, ErbB2-EGFR dimers may not be degraded once internalised. The consequence is that signalling from ErbB2 heterodimers is probably more powerful and long-lasting than from other ErbB combinations.

1.4.2.3 ErbB3 and ErbB4

ErbB3 differs from the EGFR in that, although it binds extracellular ligands, its PTK-like sub-domain lacks certain critical amino acid residues and is thus catalytically-inactive[62] (**Fig. 1**). The crystal structure of the ECD of human ErbB3 has been resolved and it displays similarities with the autoinhibited ECD of the EGFR.[46] The C-terminal region contains Tyr residues that can be phosphorylated by other ErbB family members on heterodimerisation. ErbB4 is related in sequence to other ErbB family members and its ECD is very similar to that of the EGFR. However, there are aspects that are unique.[63-65] ErbB4 has an extracellular "stalk" immediate to the N terminal to the transmembrane domain (**Fig. 1**) which results in its increased sensitivity to proteolytic cleavage by membrane metalloproteases (MMPs). This releases the ErbB4 ECD and a second proteolytic cleavage in the transmembrane domain releases the ICD from the membrane.[65,66]

1.4.3 Extracellular ligands of ErbB receptors

The four ErbB receptors are outnumbered by a multitude of extracellular polypeptide ligands that bind to one or more of the receptors (**Fig. 1**). The propeptide ligands are usually synthesised as transmembrane precursors that are subsequently proteolytically cleaved to release the soluble, active N-terminal ectodomains.[67,68] However, some may be secreted or act in a juxtacrine manner (i.e. they are cell-bound, not secreted or shed, and their action depends on intercellular contact). High affinity ErbB ligands have an EGF-like domain composed of six characteristically spaced Cys-residues which form three intramolecular

disulphide bridges and define a three-loop secondary structure. The EGF-like domain is required for binding to ErbB family receptors and for activation. EGF, transforming growth factor alpha (TGF-α), heparin-binding epidermal growth factor like factor (HB-EGF), amphiregulin, epiregulin and betacellulin bind to the EGFR. However, whereas EGF, TGF-α and amphiregulin bind exclusively to EGFR, the other three ligands (HB-EGF, epiregulin and betacellulin) also activate the ErbB4 receptor (**Fig. 1**). Epigen, the most recently described ErbB ligand,[69] binds to the EGFR but with a much lower affinity than EGF itself.[70]

A family of ligands, now known collectively as the neuregulins (NRGs) indicating their importance in the nervous system, are the products of four different genes (NRG1–NRG4) and bind to ErbB3 and ErbB4 (**Fig. 1**).[71] However, the situation is far more complex than this. For example, there are at least 15 NRG1 isoforms derived from the NRG1 gene which fall into three major subtypes (I, II or III), the classification depends on the nature of the N-terminal sequence.[72,73] These are produced by utilizing different promoters and alternative splicing. NRG1 isoforms are also variously referred to as heregulin-1, neu differentiation factors (NDFs) or acetylcholine receptor inducing activity (ARIA) (all these are Type I NRG1 isoforms); glial growth factors (GGFs) (these are Type II NRG1 isoforms); and sensory and motor neurone differentiation factors or Cys-rich domain NRG1s (these are Type III NRG1 isoforms). All three subtypes contain an EGF like domain in their glycosylated ECDs, and can exist as transmembrane proteins with ICDs and one or two hydrophobic transmembrane domains. The ECDs may be shed to produce the biologically-active species or alternatively they may remain bound and act in a juxtacrine manner. However, some are not membrane-bound but are secreted. NRG1 isoforms also contain an IgG domain (Types I and II) or a cysteine-rich domain (Type III) in the Type III-specific N terminal sequence. Less is known about NRG2 (divergent of neuregulin-1, Don-1; neural- and thymus-derived activator for ErbB kinases, NTAK) though it is known to be alternatively spliced,[74] or about NRG3 and NRG4. The dogma is that the polypeptides derived from the NRG1 and NRG2 genes bind to ErbB3 and ErbB4, whereas those from NRG3 and NRG4 bind only to ErbB4 (**Fig. 1**), although the situation may not be quite this simple.[75]

1.4.4 Activation of the EGFR

Homodimerisation or heterodimerisation of the EGFR with other ErbB family members is essential for the initiation of downstream signaling. EGFR ligands bind to a single receptor thereby inducing receptor dimerisation, rather than as originally expected,

spanning two receptor monomers. In the autoinhibited state (extracellular EGFR ligand absent), sub-domains I and III of the ECD are kept apart by the presence of an intramolecular tether between sub-domains II and IV which hold the ECD in a closed conformation (**Fig. 2**).

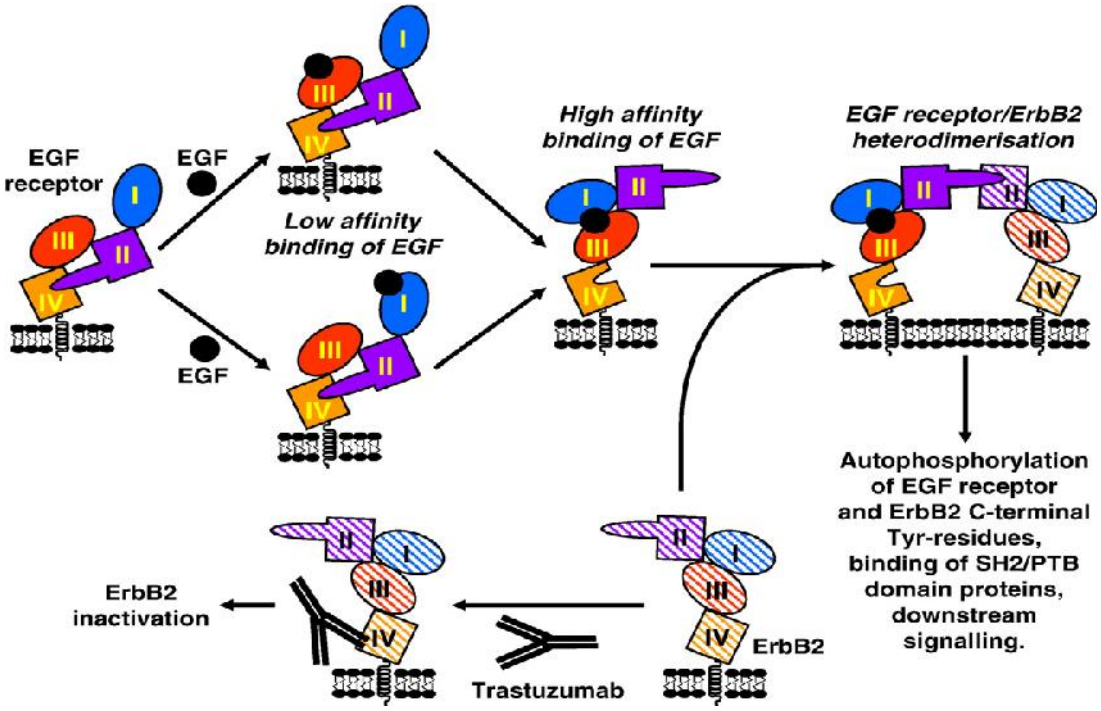


Fig. 2 *Extracellular domain rearrangement of the EGF receptor on EGF engagement, and dimerisation with ErbB2. Roman numerals refer to the sub-domains of the EGF receptor and ErbB2 ECDs.*

Ligands bind independently to either sub-domain I or sub-domain III of the ECD with low affinity, but the low affinity binding causes a change in receptor conformation. This domain rearrangement results in high affinity ligand binding to both sub-domains and in the breakage of the intermolecular tether between sub-domains II and IV. The conformational change also exposes a “dimerisation arm” present in sub-domain II (residues 242–259 in the EGFR) allowing subsequent homodimerisation or heterodimerisation and, as expected, mutations or deletions in the dimerisation arm result in a loss of ligand-mediated EGFR activation.[54,55]

Dimerisation also plays an important role in the stimulation of the intracellular PTK activity of the EGFR (**Fig. 3**). In the monomeric state, the PTK sub-domain exists as an inactive autoinhibited conformation.[49,52,53,56] On dimerisation, the PTK concentration effectively increases locally when the asymmetric PTK dimers are formed. The C lobe of one

monomer (monomer 1) binds to the N lobe of the other (monomer 2) and monomer 1 acts as an allosteric activator of monomer 2 by inducing conformational changes that force the α C

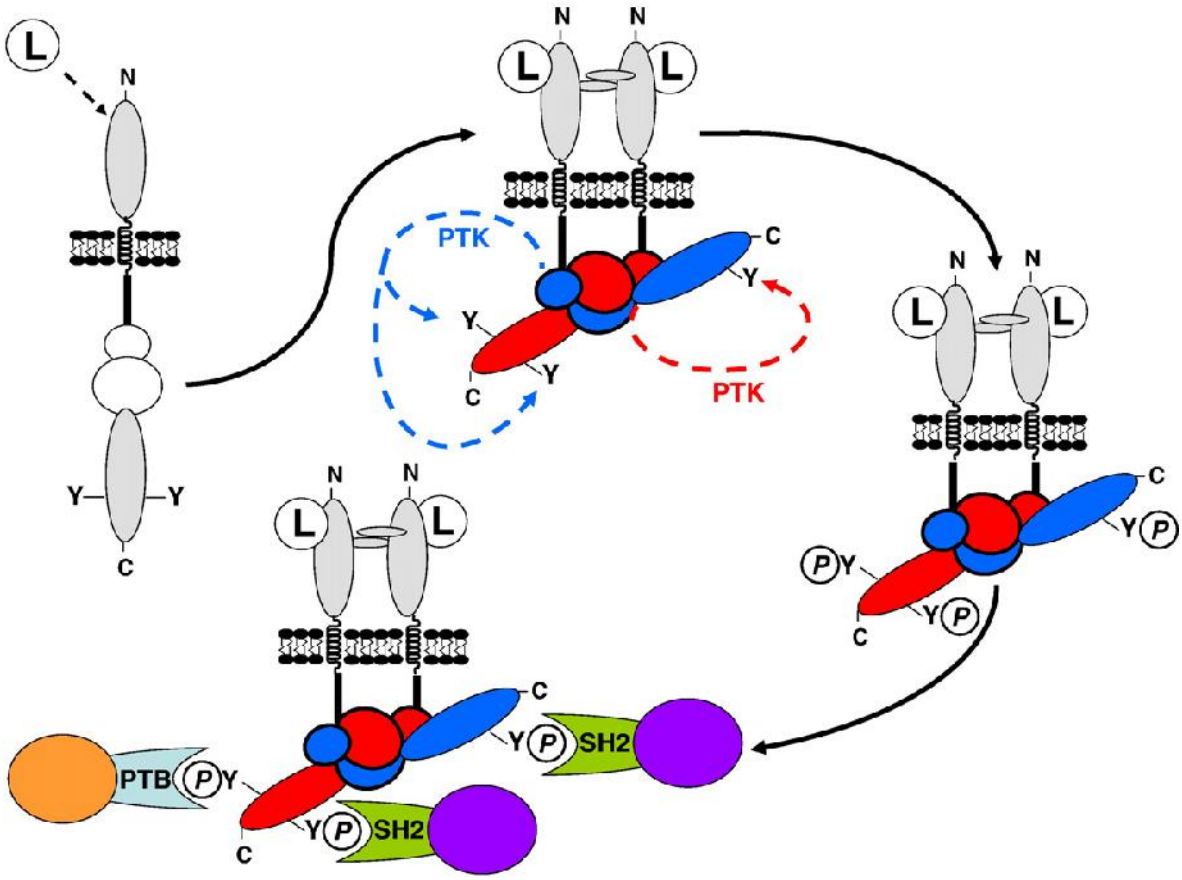


Fig. 3 *ErbB receptor dimerisation and activation. Binding of extracellular ligand (L) exposesthe dimerisation arm, receptors dimerise, and the protein Tyr-kinase (PTK) domain of receptor 1 (red) transautophosphorylates Tyr-residues (Y in the single letter code) in the C-terminal regulatory region of the intracellular domain of receptor 2 (blue) and vice versa. Subsequently, ErbB receptor effectors containing SH2 and/or PTB domains bind to the phosphotyrosine residues.*

helix of monomer 2 into the correct conformation for catalysis.[53,58] Multiple Tyr-residues in the C-terminal region of the EGFR are then trans-phosphorylated by the opposing PTK sub-domain in the receptor dimer and act as docking sites for the binding of effector proteins, with individual phosphotyrosine sites showing specificity in terms of effector binding.[70]

1.4.5 Signaling pathways of tyrosine kinase receptors

Knowledge of the cascade of biochemical events triggered by ligand stimulation of tyrosine kinase receptors has increased rapidly in recent years and provides further evidence

of the importance of these signaling pathways in cancer. In order to effectively coordinate signaling cascades, nature has created a variety of molecules known as adaptor and scaffolding proteins. These proteins play a role in intracellular signaling by both recruiting various proteins to specific locations and by assembling networks of proteins particular to a cascade. Adaptor proteins, through protein–protein interactions via specific motifs, provide a link between molecules of a signaling cascade and proteins such as Receptor Tyrosine Kina-

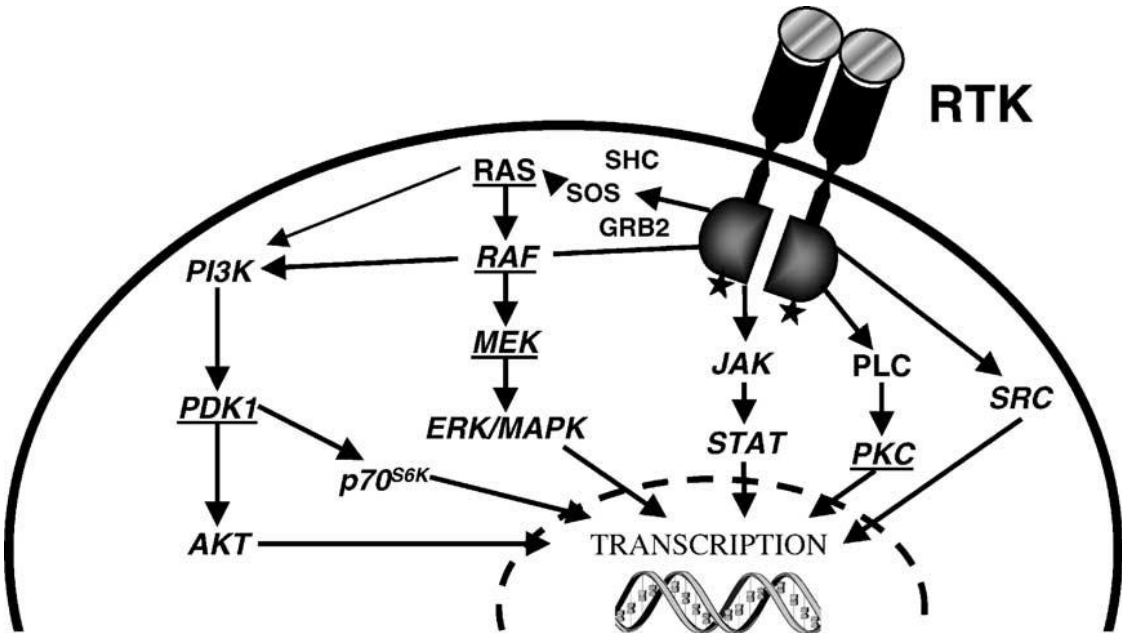


Fig. 4 *A schematic view of RTK signaling. The main pathways are indicated, with kinase names italicized. Elements of these pathways for which inhibitors are under development are also underlined. Abbreviations (pathways from left to right): PI3K, phosphatidylinositol 3-kinase; Pdk1, phosphoinositide-dependent protein kinase-1; Akt, oncogenic kinase initially isolated from a transforming mouse retrovirus (also named PKB, or related to A and C protein kinase: RAC-PK); p70S6K, ribosomal S6 kinase; Shc, (src homology collagen); Sos, (son of sevenless); Grb2, (growth factor receptor-bound protein 2) are adaptor proteins; Ras, oncogene first isolated in rat sarcomas; Raf, oncogenic kinase initially isolated from a transforming mouse virus; Mek, Map/Erk kinase (or Mkk: map kinase kinase); Erk/MapK, extracellular signal-regulated kinase/mitogen-activated protein kinase; Jak, janus kinase; Stat, signal transducer and activator of transcription; PLC, phospholipase C; PKC, protein kinase C; Src, oncogene of the chicken Rous sarcoma virus.*

ses (RTKs). One such adaptor, Grb2, is important in the activation of downstream signaling pathways such as the ras/raf/MAPK pathway. These adaptor proteins often contain a variety of motifs that mediate protein–protein interactions. Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains are protein motifs that bind to specific phosphorylated tyrosine-containing sequences, dictating particular binding partners. SH3 domains recognize and bind to proline-rich sequences in target proteins. Thus, as in the case of Grb2 which contains both SH2 and SH3 sequences, an adaptor protein can bring a cytoplasmic protein via its SH3 domain to an activated RTK via an SH2 domain-binding to phosphorylated tyrosine residues of the receptor.[76]

Recently, new signaling pathways have been described for the RTKs, in addition to the classical plasma membrane effectors which activate the ras/MAPK and PI3kinase/akt pathway (**Fig. 4**). Notably, EGFR has been shown to stimulate directly phosphorylation and nuclear translocation of signal transducer and activator of transcription (STATs) proteins. RTKs or their proteolytic fragments may also be active inside cells, and surprisingly in the nucleus where they could act directly as transcription factors.[17, 78-82]

1.4.6 Receptor internalization and trafficking

There is constant turnover of ErbB receptors at the plasma membrane as a result of both constitutive (ligand-independent) and ligand-induced endocytosis. Both processes have been studied extensively for EGFR but are less well understood for the other ErbB family members. Kinetic studies of constitutive EGFR endocytosis have shown that while the half-life for receptor internalization is approximately 30 min, the half-life for receptor recycling is much faster, approximately 5 min.[83] The kinetically faster recycling component for EGFR trafficking then predicts that 80–90% of the receptors should be localized to the plasma membrane, and this is indeed observed in cells not exposed to activating ligands.[84] Thus, the default pathway of slow internalization followed by fast recycling localizes the majority of EGFR to the plasma membrane where it is poised for activation.

Upon exposure to the ligand epidermal growth factor (EGF) the internalization rate of EGFR is accelerated 10-fold, resulting in a dramatic relocalization of receptors from the cell surface to internal compartments. This ligand-accelerated internalization is dependent upon EGFR kinase activity,[83] which has been proposed to enhance internalization through phosphorylation of the clathrin adaptor protein Eps15.[85] Ligand-induced activation of EGFR

kinase activity also recruits the E3 ubiquitin ligase Cbl leading to multiple mono-ubiquitination of EGFR, which has also been proposed to mediate receptor internalization.[86,87] However, the importance of receptor ubiquitination in internalization has been challenged,[88] and ubiquitination may play a more critical role in subsequent endosomal sorting and degradation steps. Interestingly, the accelerated ligand-induced internalization of EGFR may be unique to this member of the ErbB family as ErbB2–4 have been reported to be endocytosis impaired.[89] However, as outlined below, several negative regulators that influence ErbB trafficking and degradation are commonly absent in tumors and tumor-derived cell lines, complicating interpretations. Currently the constitutive and ligand-dependent endocytic behavior of these receptors is a matter of intense debate.[90-94]

Once internalized, either by constitutive or ligand-induced endocytosis, receptors enter the endocytic pathway where they are either rapidly recycled back to the plasma membrane through recycling endosomes, or retained in earlier structures by sorting proteins and trafficked to the lysosome for degradation. Sorting of cargo proteins at the endosome is a highly regulated process, and is a focal point for many of the proteins that regulate the stability of ErbB family members. Consequently, proteins that control the ubiquitination status of ErbB receptors help dictate steady-state levels of receptors and initiate downregulation of activated receptors.[95]

1.4.7 Defective EGFR1 activity can be caused due to many reasons

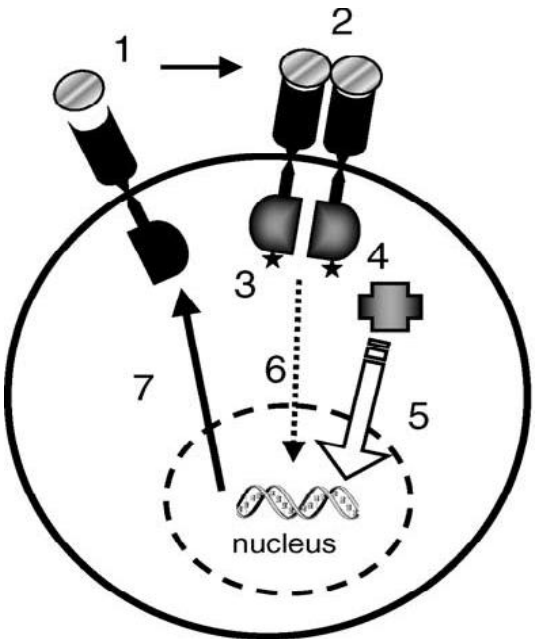
(A) Increased production of growth factors: One important process in defective EGFR1 activity is overproduction of the EGFR1 ligands like TGF-α (transforming growth factor-α) and EGF (epidermal growth factor). These growth factors are to some extent formed by the very cells that overexpress EGFR1, a situation referred to as an autocrine loop. By producing growth factors, tumor cells are thus able to stimulate their own proliferation. [96,97]

(B) Over expression of growth factor receptors: EGFR1 overexpression can occur as a result of amplification of the gene that encodes 180 kDa transmembrane glycoprotein.[98,99]

(C) Receptor activation without a ligand: Another mechanism of defective EGFR1 regulation is that of receptor activation in the absence of a ligand. This occurs, for example, in the case of EGFR1 that has been activated by mutation.[100]

1.4.8 Approaches to inhibit EGFR overexpression

Various approaches to inhibit EGFR overexpression are depicted in Fig 5.



1 : ligand binding
*Antagonists & **antibodies***
2 : receptor dimerization
Inhibitors of dimerization ?
3 : autophosphorylation
Kinase inhibitors
4 : recruitment of adaptor proteins
Inhibitors of protein-protein interactions
5 : signaling cascades
(Various inhibitors : ras, kinases)
6 : internalization and nuclear translocation
Inhibitors ?
7 : biosynthesis
Antisense, RNAi, gene therapy

Fig 5 *Different approaches to inhibit EGFR overexpression*

1.4.9 Present status of EGFR inhibitors in cancer

1.4.9.1 Monoclonal antibodies

One of the earliest approaches to EGFR blockade was the development of EGFR-specific monoclonal antibodies, which competitively inhibit ligand binding. Cetuximab (ErbituxTM, ImClone Systems/Bristol-Myers Squibb), ABX-EGF (formerly E7.6.3, Abgenix), EMD 72000 (the humanized version of EMD 55900, Merck), h-R3 [the humanized version of ior-egf/r3 (TheraCIMTM), YM BioSciences], and ICR-62 (Institute for Cancer Research) all have demonstrated antitumour activity against EGFR expressing human tumour cells in mouse xenograft models and/or in culture.[101-106]

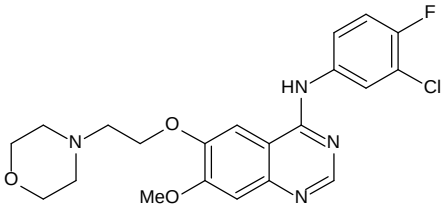
Due to the pleiotropic nature of EGFR signaling, it is not surprising that these antibodies have been shown to exert their antitumour effects by a number of different mechanisms. For example, data from numerous preclinical studies have shown that cetuximab, a human-murine chimeric anti-EGFR IgG1 antibody, inhibits cell cycle progression and cellular proliferation, promotes apoptosis, inhibits metastasis and inhibits angiogenesis. In particular up-regulation of p27Kip1, a cyclin-dependent kinase inhibitor,

appears to mediate the effects of cetuximab on cell cycle progression, while down-regulation of the angiogenic factor's vascular epidermal growth factor (VEGF) and interleukin-8 may be responsible for its effects on angiogenesis.[107-109]

The recent development of bispecific antibodies (e.g., MDX-447, Medarex) has added a new dimension to EGFR-targeted antibody therapy. MDX-447 binds simultaneously to EGFR-expressing cells and to CD64 on monocytes, macrophages, and activated neutrophils, with the aim of enhancing antibody-dependent cell-mediated cytotoxicity (ADCC) (and possibly phagocytic) effector mechanisms. MDX-447 has been shown to enhance the tumouricidal effects of CD64-bearing macrophage activated killer cells61 and is currently undergoing clinical testing in a variety of EGFR expressing cancers. An interesting variation on this theme is an anti-CD64/EGF fusion protein (H22-EGF), which is designed to act in much the same way as MDX-447. H22-EGF also has demonstrated cytotoxicity toward EGFR-expressing tumour cells in the presence of CD64-expressing cytotoxic effector cells.[110]

1.4.9.2 Small molecule inhibitors of EGFRs

Gefitinib (Iressa, 8) is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain. Gefitinib inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP) binding site of the enzyme. Thus the function of the EGFR tyrosine kinase in activating the anti-apoptotic Ras signal transduction cascade is inhibited causing inhibition of the malignant cells growth.[111]



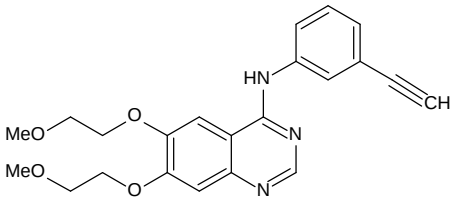
(8)

Gefitinib is currently marketed in over 64 countries. In Europe gefitinib is indicated since 2009 in advanced NSCLC in all lines of treatment for patients harbouring EGFR mutations. This label was granted after gefitinib demonstrated its utility as a first line treatment to significantly improve progression-free survival vs. a platinum doublet regime in patients harbouring such mutations. In most of the other countries where gefitinib is currently

marketed it is approved for patients with advanced NSCLC who have received at least one previous chemotherapy regime. However, applications to expand its label as a first line treatment in patients harbouring EGFR mutations is currently in process on the basis of latest scientific evidence. While gefitinib has yet to be proven to be effective in other cancers, there is potential for its use in the treatment of other cancers where EGFR over expression is involved.

As gefitinib is a selective chemotherapeutic agent, its tolerability profile is far superior to previous cytotoxic agents. Acne is reported very commonly. Other common adverse effects (≥1% of patients) include: diarrohea, nausea, vomiting, anorexia, stomatitis, dehydration, skin reactions, paronychia, asymptomatic elevations of liver enzymes, asthenia, conjunctivitis and blepharitis.[112]

Erlotinib (9) (trade name **Tarceva**) is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancers. It is a tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR).



(9)

The U.S. Food and Drug Administration (FDA) has approved it for the treatment of locally advanced or metastatic non-small cell lung cancer that has failed at least one prior chemotherapy regimen. In November 2005, the FDA approved erlotinib in combination with gemcitabine for treatment of locally advanced metastatic pancreatic cancer.[113]

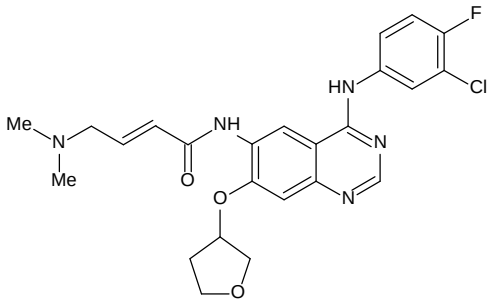
Erlotinib works only with those cancers that have an EGFR mutation, and is ineffective with other cancers. A test for the EGFR mutation in cancer patients has been developed by Genzyme Corporation. The response rate among EGFR positive patients is approximately 60%. EGFR positive patients are primarily non-smokers, light former smokers and with adenocarcinoma or subtypes like BAC. Asian females are more likely to get lung cancer even if they don't smoke, and these cancers are likely to have an EGFR mutation and be responsive to erlotinib.

Erlotinib has recently been shown to be a potent inhibitor of JAK2V617F activity. JAK2V617F is a mutant of tyrosine kinase JAK2, found in most patients with polycythemia vera (PV) and a substantial proportion of patients with idiopathic myelofibrosis or essential thrombocythemia. The study suggests that erlotinib may be used for treatment of JAK2V617F-positive PV and other myeloproliferative disorders.[114]

Tolerability profile of Erlotinib is far superior to the previous cytotoxic agents. Common side effects include rash, diarrhea, loss of appetite, fatigue and rare cases of pneumonitis.

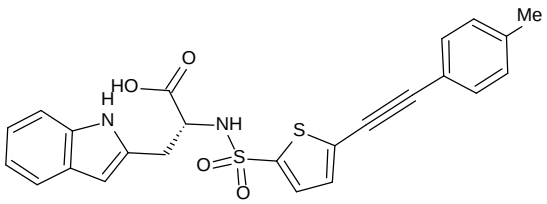
EGFR inhibitors in different stages of clinical trials

Afatinib (10, BIBW 2992) is currently in Phase II trial of genetically pre-screened cancers with EGFR and/or HER2 gene amplification or EGFR activating mutations. Moreover Phase I/II open label trial of continuous once daily oral treatment with **BIBW 2992** and **Phase** I trial in advanced non small cell lung cancer patients & Phase II trial in nonsmall cell lung cancer patients failing Erlotinib or Gefitinib showed potent inhibition. [115]



(10)

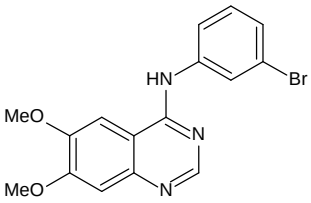
S-3304 (11) is currently in phase I/II study of the safety, pharmacokinetic interaction and efficacy in combination with standard therapy in patients with locally advanced non small cell lung cancer. [115]



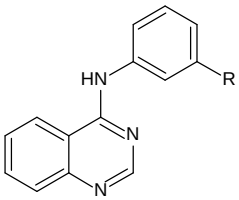
(11)

1.5 Literature review of quinazoline EGFR inhibitors

Fry *et al*. have reported that a phenylaminoquinazoline **(12)**, which was developed from leads identified through a mass screening program, was a very highly selective inhibitor of the tyrosine kinase activity of EGFR and that analogs of **12** showed competitive inhibition with respect to ATP. This finding was of particular interest because of the very high potency of **12**, which has an IC50 of 29 nM against the isolated enzyme, and 15 nM for inhibition of EGF stimulated tyrosine phosphorylation in NIH 3T3 cells. [116] Furthermore, **12** produces immediate inhibition in both isolated enzyme and cellular-based assay systems and represent a mechanistically novel class of inhibitors of EGFR. [117]



(12) PD 153035



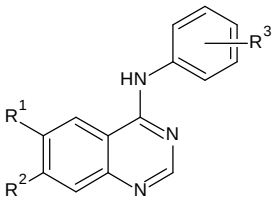
(13) R = Cl

(14) R = Br

Rewcastle *et al*. have reported a series of 4-substituted quinazolines and related compounds, as inhibitors of EGFR-TK. In the 4-anilino series, substitution on the 3-position of the phenyl ring with small lipophilic electron-withdrawing groups were beneficial, the 3-C1 and 3-Br derivatives **(13** and **14)** being the most potent (IC_{50} 23 and 27 nM against isolated EGFR-TK respectively) derivatives provided analogs with enhanced potency. They have studied two series of compounds [4-(phenylmethyl)amino and 4-(3-bromophenyl) amino] to determine SARs for quinazoline substituents. In the more active 4-(3-bromophenyl)amino series, electron-donating groups (NH_2, OMe) at the 6- or 7-positions increased activity, in a pattern consistent with a requirement for high electron density in the vicinity of the 8-position of the quinazoline ring. The 6,7-dimethoxy derivatives were the most effective in both series. [118]

Bridges *et al*. have reported a series of 4-anilino- and 4-benzylamino-substituted quinazolines as potent reversible inhibitors of EGFR tyrosine kinase. The two side chains are equipotent in the parent quinazoline ring. Addition of a 7-methoxy group is modestly favorable in both the series and the 6,7-dimethoxy analogs are more active, benzyl being the best, with an impressive 10 nM IC_{50} against the enzyme. However, when the aromatic side

chain nucleus was substituted, the anilines were capable of great enhancements in activity as exemplified by compound **(15)** having 3-bromo substituent[12,19] , whereas the benzylamino side chains in this series[12] and a closely related series[15], revealed that nuclear substitution on the benzylic side chain was almost always detrimental.[119]



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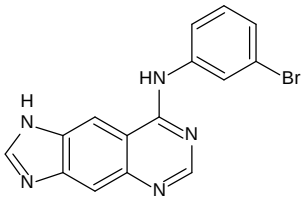
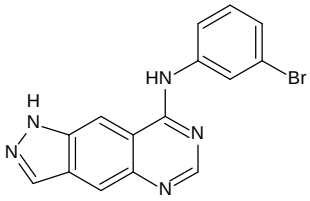
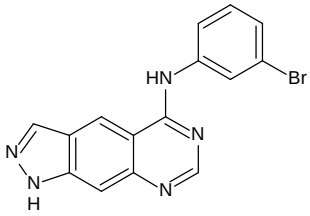


(**16**)



(**17**)

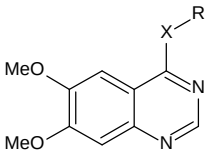


(18)

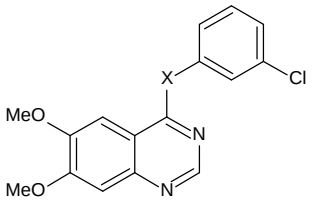
Following the discovery of 4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline **(12)** as an extremely potent (IC_{50} 0.025 nM) inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), Rewcastle e*t al*. have prepared several fused tricyclic quinazoline analogs and evaluated them for their ability to inhibit the enzyme. The most potent compound was the linear imidazo[4,5-*g*]quinazoline (**16**), which exhibited an IC_{50} of 0.008 nM for inhibition of phosphorylation of a fragment of phospholipase C-γ1 as substrate. While N-methyl analogs of **16** showed similar potency, analogous N-[2-(dimethylamino)ethyl] derivative was less effective. The next most potent compounds were the linear pyrazoloquinazolines (**17** and **18**) (IC_{50}s 0.34 and 0.44 nM) and pyrroloquinazoline (IC_{50} 0.44 nM), while several other linear tricyclic ring systems of similar geometry to **16** (triazolo-, thiazolo-, and pyrazinoquinazolines) were less effective. In the imidazo[4,5-*g*]quinazoline and pyrroloquinazoline series, the corresponding angular isomers were also much less effective than the linear ones. These results are consistent with structure-activity relationship studies previously developed for the 4-[(3-bromophenyl)amino]quinazolines, which suggested that small electron-donating substituents at the 6- and 7-positions were desirable for high potency. Cellular studies of the linear imidazoloquinazoline **(16)** show that

it can enter cells, and rapidly and very selectively shut down EGF-stimulated signal transmission by binding competitively at the ATP site of the EGFR. [120]

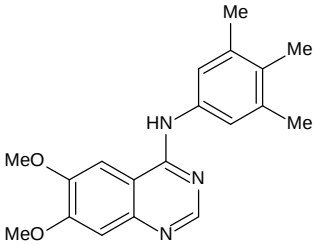
Myers *et al*. have reported a series of 4-(anilinophenoxy), and 4-(thiophenoxy)quinazolines; replacement of NH (IC_{50} of 0.03 µM) either with O (IC_{50} of 0.02 µM) or S (IC_{50} of 0.01 µM) retain potency against EGFR tyrosine kinase. However, replacement of NH with NCH_3 (IC_{50} of 0.1 µM) reduces the EGFR potency. This loss of potency is likely due in part to the conformation of the phenyl group in relation to the quinazoline ring and not to the loss of a specific NH interaction since the oxygen and sulfur analog retain potent activity against EGFR. The 3-chlorophenoxy and 3-chlorothiophenoxy derivatives **(19** and **20)** were extremely potent EGF-R inhibitors. [121]



(19, X= NH, O, S**)**



(20, X= O, S**)**



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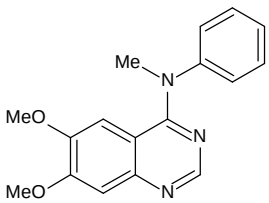
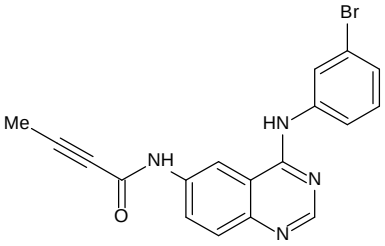


(22)

An accompanying report describes the preliminary SAR study of RPR-108518A **(21)**, an inhibitor of $p56^{lck}$ tyrosine kinase activity[122,123]. Myers *et al*. have screened several analogs in this series for EGFR, PDGFR and CSF-IR inhibitory activities[121]. The N-methyl analog RPR-108514A **(22)** was found to be a relatively weak inhibitor of EGFR and was essentially inactive towards $p56^{lck}$. However, RPR-108514A **(22)** was found to be a potent and relatively selective inhibitor of CSF-1R. Based on their study they have proposed SAR for this series. Simple substituents on the phenyl ring resulted in compounds with diminished activity relative to compound RPR-108514A **(22)** [124]. The optimum activity was seen with the methyl substituent at the 3-position. Replacement of the 6,7-dimethoxy groups on the quinazoline with one or two hydrogens was found to be deleterious. In general, other

substituents on the quinazoline ring were not well-tolerated In particular, substitution at the 2- or 8-positions of the quinazoline ring completely eliminated activity, suggesting that there was an important binding interaction between the enzyme and the nitrogen at the 1-position of quinazoline. This SAR is consistent with that observed in the series of quinoline-based inhibitors of PDGFR and quinazoline-based inhibitors of both p56[1ck] and EGFR.[122, 123 & 125]

It has been shown previously that 4-anilinoquinazolines compete with the ability of ATP to bind the epidermal growth factor receptor (EGF-R), inhibit EGF-stimulated autophosphorylation of tyrosine residues in EGFR, and block EGF-mediated growth[126]. Since millimolar concentrations of ATP in cells could reduce the efficacy of 4-anilinoquinazolines in cells and the activity of these compounds would not be sustained once they were removed from the body[127]. Hence, Discafani *et al*. reasoned that irreversible inhibitors of EGFR might improve the activity of this series of compounds in animals. Molecular modeling of the EGFR kinase domain was used to design irreversible inhibitors. The most potent compound found was **23**. This compound was covalently bound to EGFR. It also specifically inhibited kinase activity of the protein (IC_{50} 370 ± 120 pM), blocked EGF-stimulated autophosphorylation of the receptor in cells (IC_{50} 5 nM), inhibited cell proliferation (IC_{50} 31–125 nM) primarily in a cytostatic manner in cell lines that overexpressed EGFR or c-ErbB-2, and profoundly blocked the growth of a tumor that overexpressed EGFR in nude mice (when given orally at 80 mg/kg/day for 10 days, daily). [128]



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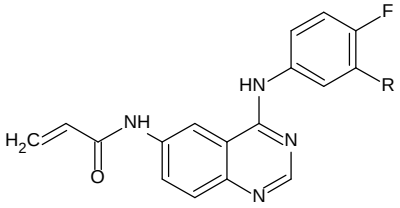


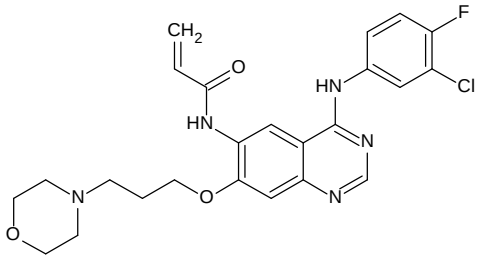
(24) R = Cl

(24A) R = Br

Smaill *et al*. have synthesized and reported biological activity of a range of 6- and 7-substituted acrylamidoquinazolines and acrylamidopyrido[*d*]pyrimidines. They all showed irreversible inhibition and in general similar potencies in the isolated enzyme assay; the best dual inhibition potencies were shown by compounds **(24)** (IC_{50} 0.69 and 2.7 nM against isolated EGFR and ErbB2 TK respectively) and **24A** (IC_{50} 0.75 and 3.1 nM against isolated EGFR and ErbB2 TK respectively).[129] The quinazolines were generally less potent against

ErbB2 than EGFR in the cellular assays, but the pyridopyrimidines were equipotent in both assays, providing an interesting class of generic inhibitors of the ErbB family. The compounds showed encouraging *in vivo* activity, being cytostatic rather than cytotoxic, but with good therapeutic indices. [130]

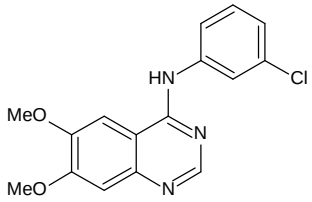
Smaill *et al*. have synthesized 4-anilinoquinazoline- and 4-anilinopyrido[3,2-*d*]pyrimidin-6-acrylamides substituted with solubilizing 7-alkylamine or 7-alkoxyamine side chains. Quinazoline analogs with 7-alkoxyamine solubilizing groups were potent irreversible inhibitors of the isolated EGFR enzyme, with IC_{50} values ranging from 2 to 4 nM that potently inhibited both EGF-stimulated autophosphorylation of EGFR in A431 cells and of heregulin-stimulated autophosphorylation of ErbB2 in MDA-MB 453 cells.[131] 7-Alkylamino- and 7-alkoxyaminopyrido[3,2-*d*]pyrimidines were also irreversible inhibitors with equal or superior potency against the isolated enzyme but were less effective in the cellular autophosphorylation assays. The quinazolinepropoxymorpholide **(25)** showed excellent *in vivo* antitumor activity, giving growth delays in A431 xenografts exceeding 50 days following oral administration. This compound (as the dihydrochloride salt, **CI 1033**) has been selected for clinical evaluation.[132]



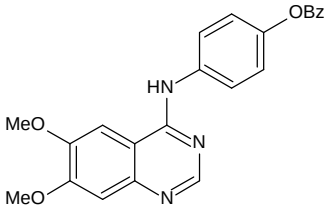
(25)

Screening identified quinazoline **4557W (26)** as a potent inhibitor of both c-ErbB-2 and EGFR [c-ErbB-2 0.079 µM, EGFR 0.020 µM (isolated enzyme), 2.0 µM in HB4aC5.2 cells, 1.2 µM in BT474, both overexpressing c-ErbB-2; 2.5 µM in HN5 cells overexpressing EGFR]. This combined EGFR/c-ErbB-2 potency was in direct contrast to smaller, previously reported anilinoquinazolines like **CAQ**[133,134] **(27)** and subsequent analogs which had demonstrated selectivity for EGFR over c-ErbB-2. [135-138] S. Cockerill *et al*. have constructed a binding hypothesis for this compound utilizing knowledge of P38 binding based upon the principle of quinazoline binding through N1 to Thr798, the residue equivalent to Met109. [139] This binding hypothesis has the benzyloxyaniline accommodated in the back of the hydrophobic pocket with the 6,7-dimethoxy groups pointing towards the lip of the ATP

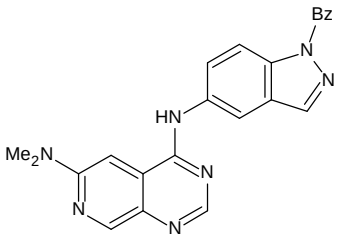
binding cleft. They decided to investigate the effect of this proposed hydrophobic binding interaction by imposing a conformational restriction into the aniline fragment. A bicyclic nucleus was chosen to replace the aniline system and reduce the number of rotatable bonds in this region from 3 to 2. Subsequently they selected and screened a range of bicyclics. Greater intrinsic potency was observed for 6,7-dimethoxyquinazolines and pyridopyrimidines, in line with those previously reported for EGFR inhibition[6,7]. In general, 1-substitution of the bicyclic anilino system was very potent. However in the case of benzimidazole, 2-substitution was clearly the preferred regioisomer. Although the precise reason for this remains unclear, one can speculate the potential H-bonding effect of Lys53 (the equivalent residue in c-ErbB-2 is Lys753) compensating for a subsequent conformational alteration in the hydrophobic region of the binding pocket. Of particular interest from this evaluation was the indazolyl compound **GW974 (28)** (IC_{50} of 0.018 µM against c-ErbB-2 and IC_{50} of 0.001 µM against EGFR isolated enzyme), that showed excellent dual inhibition activity against both EGFR and c-ErbB-2. [139]



(26) 4557W



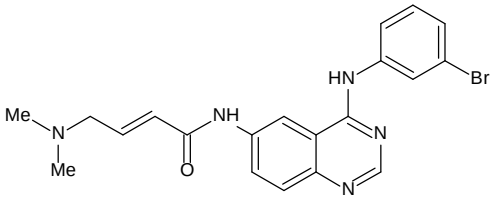
(27) CAQ



(28) GW974

Tsou *et al*. have reported a series of new 6-substituted 4-(3-bromophenylamino)quinazoline derivatives as irreversible inhibitors of EGFR and HER-2 kinases. These inhibitors have, at the C-6 position, butynamide, crotonamide, and methacrylamide Michael acceptors bearing water-solublilizing substituents. They have shown that attaching a basic functional group onto the Michael acceptor results in greater reactivity due to intramolecular catalysis of the Michael addition and/or increased electrophilicity of the double bond. This, along with improved water-solubility, results in

compounds with enhanced biological properties. Most of the compounds inhibit both EGFR and HER-2 kinases in nanomolar range. One of the compounds **(29)** showed excellent oral activity in a human xenograft model of cancer. [140]



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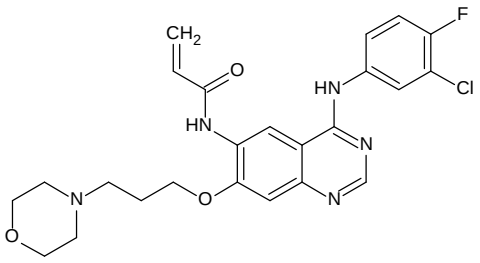
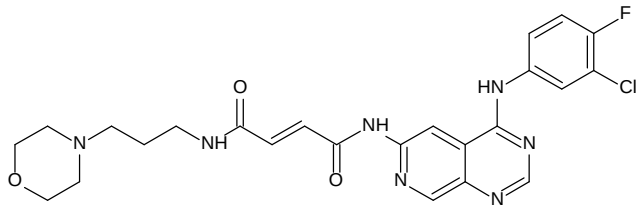


(30) Canertinib, CI-1033

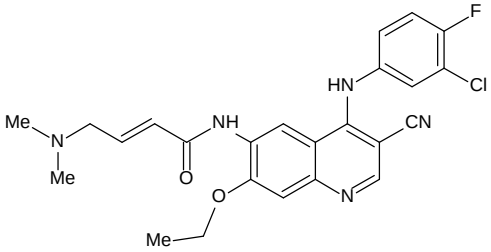
In expectation that the high levels of intracellular ATP in some cell lines may make it difficult to achieve sufficiently high intracellular levels of EGFR inhibitors to shut-down EGF-stimulated autophosphorylation for long periods, Smaill *et al.*[141] have been exploring the use of irreversible inhibitors. They have recently reported[144,13-15] that 6- and (to a lesser extent) 7-acrylamide analogs of the 4-anilinoquinazolines and pyrido[*d*]pyrimidines act at the ATP binding domain of EGFR, specifically alkylating an adjacent Cys-773 residue and irreversibly shutting down kinase activity. The 6-acrylamides are irreversible inhibitors of both EGFR and ErbB2 autophosphorylation and show significantly improved *in vivo* antitumor activity compared to closely related reversible analogs[13]. They tolerate a wide range of structural variations in the molecule with retention of irreversibility and potency, including substitution at the vacant 7-position of the quinazoline nucleus with a range of amine-bearing side chains, and provide a class of soluble, orally active, potent, selective, and irreversible inhibitors of the EGFR family of tyrosine kinases; among them **30 (CI-1033)** being the most potent has been shown to irreversibly inhibit the EGFR tyrosine kinase with a remarkable IC_{50} value of 1.5 nM[141-144].

Attempts to develop a soluble pyrido[*d*]pyrimidine analogs of **30**, by introducing amine-bearing soluble side chains at the 7-position of a pyrido[3,2-*d*]pyrimidine nucleus, Smaill *et al.* met with only limited success[145]. While these compounds showed excellent potency for inhibition of isolated EGFR enzyme, they were considerably less potent in cellular assays when compared to the analogous quinazolines. This lack of potency has in part been attributed to the increased reactivity of the acrylamide moiety toward cellular glutathione. [145]

In further development of this class, Smaill *et al.* have reported the synthesis and biological activity of a range of 4-anilinoquinazolines and pyrido[3,4-*d*]pyrimidines substituted at the 6-position with a variety of Michael acceptors apart from the parent acrylamide. These results show that a range of Michael acceptors, apart from the unsubstituted acrylamide at the 6-position of 4-anilinoquinazolines and pyrido[3,4-*d*]pyrimidines, provide irreversible inhibitors of the EGFR enzyme. Of the non-acrylamide Michael acceptors studied, only the vinylsulfonamide provided comparably potent irreversible inhibitors, but these were less stable. Within the modified acrylamides, there was very limited bulk tolerance for substitution at the acrylamide nitrogen, with only the N-methyl analog of 4-anilinopyrido[3,4-*d*]pyrimidines retaining irreversible activity, and there was no tolerance at all for substitution at the acrylamide α-carbon.[146] In contrast, quite large number of electron-withdrawing groups (which increase acrylamide electrophilicity) were acceptable at the α-carbon. These amide-derived soluble analogs were potent irreversible inhibitors of isolated EGFR and effective inhibitors of both EGF-stimulated autophosphorylation of EGFR and heregulin-stimulated autophosphorylation of ErbB2 in cellular assays, with activity profiles comparable to that of the clinical agent **30**.[147] However, the best compound **(31)** (IC_{50} of 0.76 nM against the isolated EGFR and IC_{50} of 2.4 nM against the enzyme from A431 vulval squamous carcinoma cells) of these was not nearly as active as **CI-1033 (30)** *in vivo*.[147] Thus positioning the solubility enhancing group of the α-carbon of the acrylamide might not be as useful as positioning it separately off the 7-position, because it might raise the general alkylating ability of the inhibitor.[148]



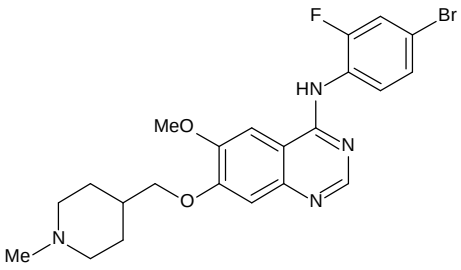
(31)



(32) EKB-569

Based on modeling studies, from the 4-anilinoquinazolines Wissner *et al* have designed and synthesized a series of 4-anilinoquinoline-3-carbonitriles as EGFR inhibitors[149]. Ultimately, this series was optimized by this group resulting in the irreversible binding EGFR kinase inhibitor **(32)** (IC_{50} of 0.083 &1.229 µM for isolated EGFR & HER-2 respectively; IC_{50} of 0.03 & 0.007nM for A-431 & SKBR3 cell lines). [150]

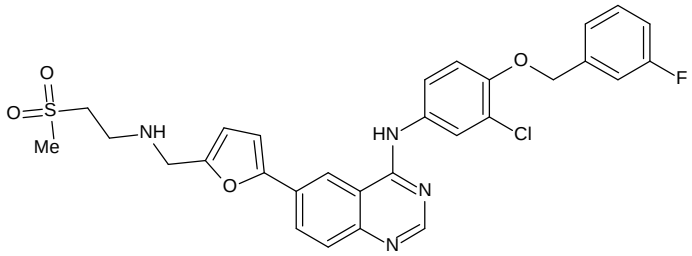
Hennequin *et al*. have reported a novel subseries of 4-anilinoquinazolines that possess basic side chain at the C-7 position of the quinazoline nucleus. This subseries contains potent, nanomolar inhibitors of KDR (median IC_{50} 0.02 µM, range 0.001-0.04 µM), which are comparatively less potent vs Flt-1 tyrosine kinase (median IC_{50} 0.55 µM, range 0.02-1.6 µM) [151,152]. The compounds also retain some inhibitory activity against the tyrosine kinase associated to the endothelial growth factor receptor (EGFR) (median IC_{50} 0.2 µM, range 0.075-0.8 µM) but demonstrate selectivity vs FGF receptor 1 (median IC_{50} 2.5 µM, range 0.9-19 µM). This selectivity profile is also evident in a growth factor-stimulated human endothelial cell (HUVEC) proliferation assay (i.e., inhibition of VEGF > EGF > FGF), with inhibition of VEGF-induced proliferation being achieved at nanomolar concentrations (median IC_{50} 0.06 µM). Further examination of compound **(33, ZD6474)** in recombinant enzyme assays revealed excellent selectivity for the inhibition of KDR tyrosine kinase (IC_{50} 0.04 µM) vs the kinase activity of ErbB2, MEK, CDK-2, Tie-2, IGFR-1R, PDK, PDGFRâ, and AKT (IC_{50} range: 1.1 to >100 µM). Anilinoquinazolines possessing basic C-7 side chains exhibited markedly improved aqueous solubility over previously described anilinoquinazolines possessing neutral C-7 side chains (up to 500-fold improvement at *p*H 7.4). Oral administration of representative compounds to mice (50 mg/kg) produced plasma levels between 0.2 and 3 µM at 24 h after dosing. The authors demonstrated a very attractive *in vitro* profile combined with excellent solubility (330 µM at *p*H 7.4) and good oral bioavailability in rat and dog (>80 and >50%, respectively). This compound demonstrated highly significant, dose-dependent, antitumor activity in athymic mice. Once daily oral administration of 100 mg/kg of compound **(33)** for 21 days inhibited the established Calu-6 lung carcinoma xenografts by 79% ($P < 0.001$, Mann Whitney rank sum test), and substantial inhibition (36%, $P < 0.02$) was evident with 12.5 mg/kg/day. [153]



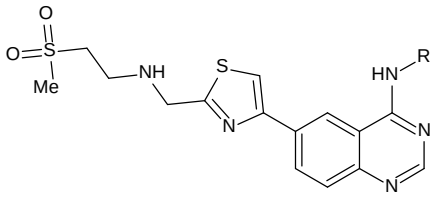
(33) ZD6474

The tolerance for substituents in the 6-position of the quinazoline was generally large for potent enzyme inhibition, so Gaul *et al*. used cellular activity to develop the SAR to determine the preferred substituents. They had demonstrated that one of the best 6-position

side chain substituents for providing optimal cellular activity was achieved when the 2-(methylsulfonyl)ethylamino group was the preferred side chain substituent linked via a methylene unit to a heterocyclic ring as in **34 (GW572016)**[154]. The quinazoline scaffold provides the necessary binding properties for inhibition of the ErbB family of tyrosine kinases[155]. Smaller aniline substituents generally provide compounds with potent EGFR TK inhibition, while larger aniline substituents generally confer greater dual ErbB-2 and EGFR tyrosine kinase inhibition. Using a mix and match strategy for lead optimization, the 'best' anilines for dual inhibition were combined with the optimized side chain to synthesize a series of 6-(2,4-thiazolyl)quinazoline derivatives. The best overall cellular activity is observed with compounds [**35** (N-1-benzylindazole, average IC_{50} 0f 0.012 µM) and **35A** (4-[3-fluorobenzyloxy]-3-chloroaniline, average IC_{50} 0f 0.012 µM) against EGFR & ErbB-2 TK]. The most promising 6-thiazolylquinazoline compounds **(35 & 35A)** exceeded 100-fold selectivity for tumor cells over normal cells and afforded better oral exposures than the **GW572016 (34)** in the mouse model.[156-159]



(34) GW572016, Lapatinib



(35) R= N-1-Benzylindazole

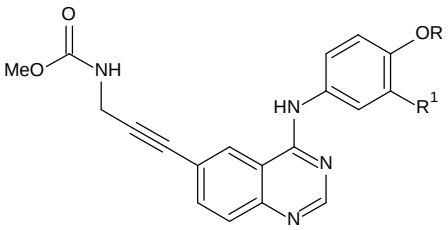
(35A) R= 4-(3-Fluorobenzyloxy)-3-chloroaniline

To investigate whether changes to the 4-anilino functionality would indeed provide the ability to significantly alter selectivity between EGFR and ErbB2 kinases, a series of 6-alkynyl-4-anilinoquinazolines was prepared by Bhattacharya *et al*. in which the 6-alkynyl quinazoline remained constant and the 4-anilino group was altered. Derivatives with small, non-polar substituents at the aniline 3-position such as ethynyl, methyl or bromo all provide exceptional potency against EGFR kinase, but showed 80–100 times less activity against ErbB2 kinase. These results are consistent with the reported potency and selectivity of the EGFR inhibitors erlotinib **(9)** and PD-153035 **(12)**. [160]

Compound **(36)** in which the small substituent at the aniline 3-position is removed and replaced with a large lipophilic phenyl ether at the aniline 4-position, showed no activity

against EGFR while ErbB2 inhibition was slightly enhanced. This trend was also observed for quinazoline inhibitors bearing similar 4-anilino functionality. [161]

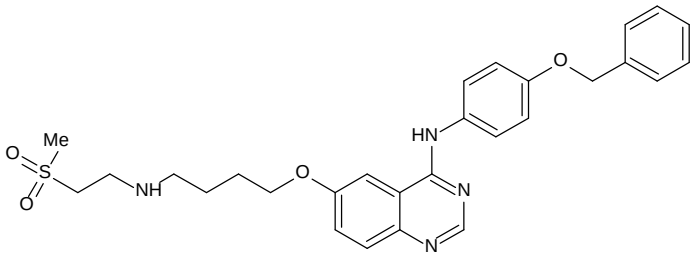
Further refinement of the potency and selectivity profile of **36** was achieved by the incorporation of both a phenyl ether at the aniline 4-position and a small non-polar substituent such as methyl or chloro at the aniline 3-position. While the nature and relative positioning of functionality around the aniline ring itself provide dramatic changes to ErbB2 potency and selectivity, changing the terminal phenyl ether to a 3′-pyridyl ether provides additional improvement in ErbB2 activity with a concomitant reduction in EGFR activity as observed in compounds **(36A and 36B)**. Both the compounds **(36A)** (IC_{50} 0f 42 & 4200 nM; against EGFR & ErbB-2 TK respectively) and **(36B)** (IC_{50} 0f 55 &7000 nM; against EGFR & ErbB-2 TK respectively) inhibit ErbB2 kinase. [160]



(36) R= Phenyl, R^1= H

(36A) R= 3′-Pyridyl, R^1= Methyl

(36B) R= 3′-Pyridyl, R^1= Chloro**)**



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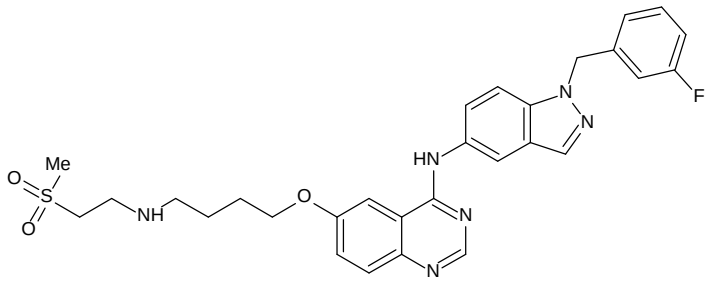
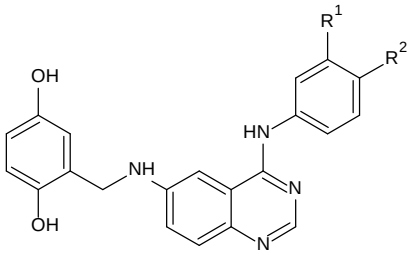


(38)

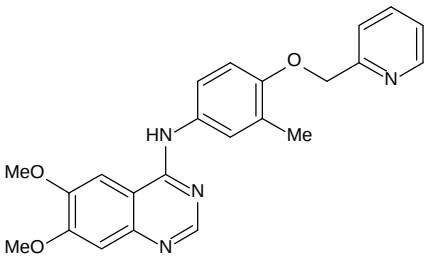
A series of 6-alkoxy-4-anilinoquinazolines derivatives with a variety of C-4 anilines and various C-6 ether linkers as ErbB2/EGFR TK inhibitors were prepared by Zhang *et al*. The best compound among various C-6 ether linkers was **37** having excellent dual inhibitor enzyme profile (IC_{50} EGFR: 74 nM, ErbB2: 95 nM). The most potent compound of various C-4 anilines was **38** (IC_{50} EGFR: 24 nM, ErbB2: 19 nM). [161]

The results of Hodge and Traxler revealed a bioisosteric relationship between the salicylic group and the quinazoline,[162,163] Albuschat *et al*. have synthesized the 4-anilinoquinazolines **(39 and 39A)** with a 6-[(2,5-dihydroxybenzyl)amino] side chain**.** The 4-anilinoquinazolines **(39 and 39A)** are relatively potent EGFR tyrosine kinase inhibitors with approximate IC_{50} values in the range of 0.1–1 μM. Additionally, both substances cause antiproliferative to cytotoxic effects on certain human cancer cell lines. Worthy of remark is, that **39B** showed excellent cytotoxic activity on the leukemia cell line CCRFCEM with GI_{50} and TGI values at the 0.01 μM levels (0.04 and 0.09 μM) and a LC_{50} value of 33.7 μM. [164-167]



(39) R^1=Br, R^2=H

(39A) R^1=Cl, R^2=F



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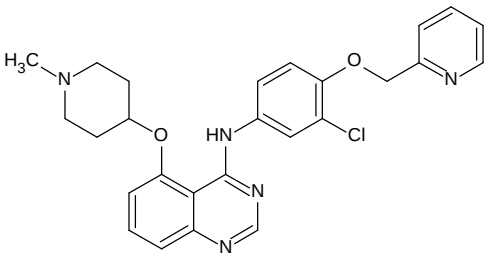
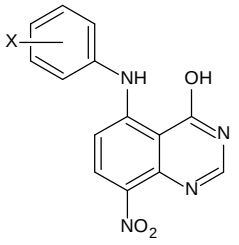


(41)

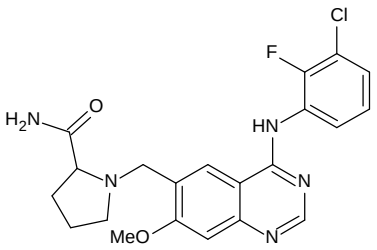
Ballard *et al*. found 6,7-substituted quinazoline[168] **(40)** to be a moderately potent inhibitor of ErbB2 receptor tyrosine kinase. The extended aniline motif was assumed to interact with the selectivity pocket in the ErbB2 active site[167]. From consideration of an in-house homology model and related work on inhibitors of c-Src[168], they reasoned that switching of the quinazoline substituent from the 6- to the 5-position could improve ErbB2 affinity through occupation of the kinase sugar pocket. They optimized 5-substituted quinazolines containing an extended aniline motif that led to potent and selective inhibitors of ErbB2 receptor tyrosine kinase, and a representative compound **(41)** having IC_{50} 0.002 & 0.14 μM against ErbB2 & EGFR isolated enzymes and inhibited tumour growth in a mouse xenograft model. [168]

Jin *et al*. have synthesized and assessed a series of novel 5-substituted 4-hydroxy-8-nitroquinazolines that may function as inhibitors of EGFR/ErbB-2-mediated cellular signaling pathways. The SAR was discussed in terms of the inhibitory activity against the proliferation of two human carcinoma cell lines known to express high levels of EGFR and ErbB-2. For the new 5,8-disubstituted quinazoline derivatives, the 5-anilino substituent is essential for its activity. Furthermore, substituent on the 5-anilino portion has a substantial effect on the potency and selectivity of the resulting compound with respect to the inhibition of EGFR and ErbB-2 receptors. The 5-anilinoquinazoline **(42)** was a selective inhibitor of EGFR, whereas the 5-(3-chloroanilino)quinazoline **(42A)** displayed selective inhibition of ErbB-2. More significantly, two potent dual inhibitors **(42B** and **42C)** of EGFR and ErbB-2 were discovered with the alkoxy substituents at the 4′-position of 5-anilino group. This study was the first attempt to identify new structural types of EGFR/ErbB-2 signaling inhibitors, by incorporation of the anilino group at the 5-position of 4-hydroxy-8-nitroquinazoline core structure, providing promising new templates for further development of potent inhibitors targeting both EGFR and ErbB-2 tyrosine kinases. [169]



(42) X= H **(42B)** X= 4-OMe

(42A) X= 3-Cl **(42C)** X= 4-OCH_2Ph



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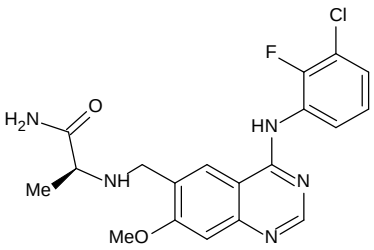
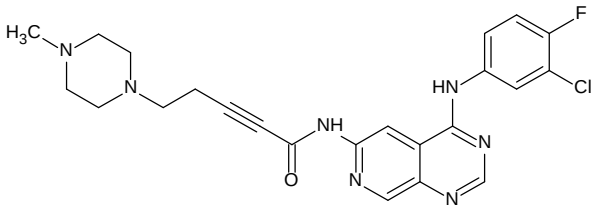


(44)

The structure–activity relationship of a novel subseries of 4-anilinoquinazoline EGFR inhibitors substituted at the C-6 position with carbon-linked side chains has been investigated by Hennequin *et al*. This exploration has led to the discovery of novel aminomethyl carboxamides **43** (IC_{50} 0.03 & 0.055 µM on isolated EGFR & KB cell lines respectively) & **44** (IC_{50} 0.008 & 0.025 µM, isolated EGFR & KB cell line respectively). They displayed excellent physical properties and high bioavailability in rat and dog *in vivo*. Further, they showed significant antitumour activity in xenograft model. [170]

Klutchko *et al*. have synthesized and developed structure-activity relationships for inhibition of ErbB1, ErbB2 and ErbB4 for a series of alkynamide analogs of quinazoline- and pyrido[3,4-*d*]pyrimidine-based compounds. The compounds showed pan-ErbB enzyme inhibition but were on an average about 10-fold more potent against ErbB1 than against ErbB2 and ErbB4. For cellular inhibition, the nature of the alkylating side chain was an important determinant, with 5-dialkylamino-2-pentynamide type Michael acceptors providing the highest potency. This is suggested to be due to an improved ability of the amine to participate in an autocatalysis manner of the Michael reaction with enzyme cysteine residues[172]. Pyrido[3,4-*d*]pyrimidine analog **(45)** was selected for *in vivo* evaluation and achieved tumor regressions at 10 mg/kg in the A431 human epidermoid carcinoma and at 40 mg/kg for the SF767 human glioblastoma and the SKOV3 human ovarian carcinoma. Complete stasis was observed at 40 mg/kg in the BXPC3 human pancreatic carcinoma as well as in the H125 human non-small-cell lung carcinoma. [171,72]

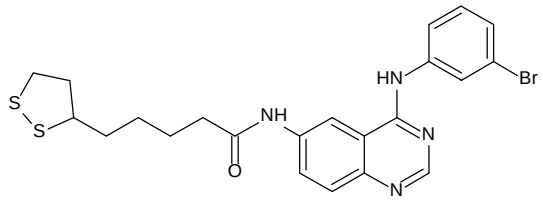


(45)

The multifactorial mechanistic nature of cancer cells requires development of multifunctional therapeutic tools, i.e., single compounds able to interact with multiple altered pathogenetic pathways. Following this rationale, Antonello *et al*. have synthesized compounds **(46-46B)**, by combining the structural features of the EGFR inhibitor **PD153035 (12)** and lipoic acid, which among other therapeutic effects triggers apoptosis in human cancer cells. Compound **(46B)** was able to completely block the phosphorylation induced by EGFR-TK, with an IC_{50} 0.060 ± 0.009 µM, whereas homologues **(46** and **46A)** were only weak inhibitors. These compounds have IC_{50} ranging from 7.90 µM (**46B**) to 21.56 µM (**46**), with potency highly dependent on the substituent at the 6-position of the anilinoquinazoline nucleus. [173]

Wissner *et al*. have prepared a series of quinazoline derivatives containing 6-(4-dimethylamino)crotonamide Michael acceptor group that targets Cys-773 in EGFR and/or a 4-amino[1,4]benzoquinone moiety that targets Cys-1045 in VEGFR-2. It is instructive to compare compounds having a quinone ring but no Michael acceptor group **(47-47B)**, with the respective compounds having the same quinone substituents and also having the Michael

acceptor group **(48-48B)**. While the former compounds are potent VEGFR-2 inhibitors with IC_{50} values in the range of 46.1–53.7 nM at 1 μM ATP, they lack the ability to inhibit EGFR significantly. In contrast, **(48-48B)** are relatively potent inhibitors of both the enzymes. Similarly, the compounds that have a Michael acceptor but do not have the quinone ring (**32) (EKB-569)** are potent inhibitors of EGFR, but very weak inhibitor of VEGFR-2[174].



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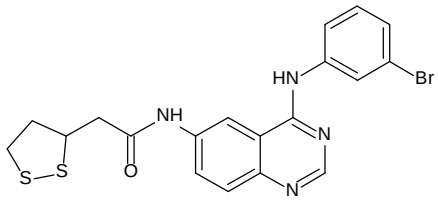
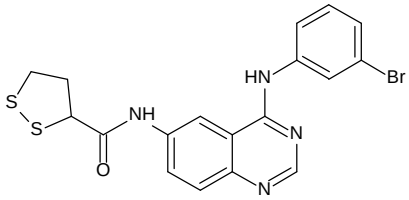
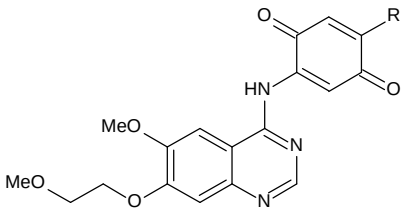


(46A)



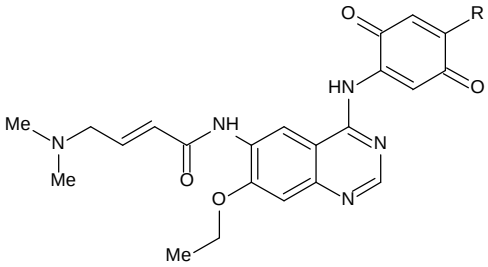
(46B)



(47) R = OCH_2-3-C_6H_4-OCH_3

(47A) R = OCH_2-C_6H_5

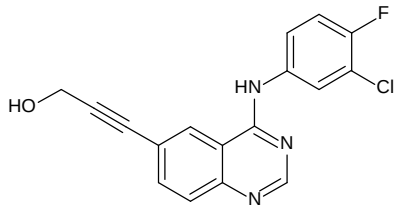
(47B) R = OCH_2-3-C_6H_4-F



(48) R = OCH_2-3-C_6H_4-F

(48A) R = OCH_2-C_6H_5

(48B) R = OCH_2-3-C_6H_4-OCH3

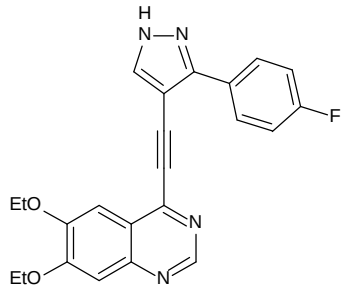


(49)

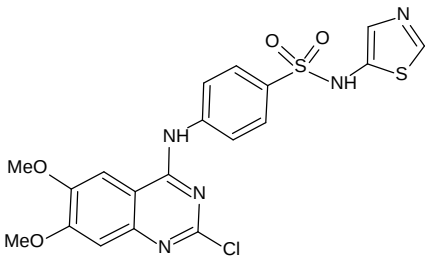
Liu *et al*. reported a series of 4-anilinoquinazoline derivatives with various alkynyl groups at C-6 or C-3′ positions prepared via Sonogashira reaction of the corresponding bromo-substituted 4-anilinoquinazolines. Bioactive assay of these compounds for the *in vitro* EGFR kinase inhibition demonstrated that 6-hydroxypropynyl-4-anilinoquinazoline **(49)** was

a very potent EGFR kinase inhibitor with an IC_{50} of 14 nM. It is noticeable that compound **(49)** exhibited even more potent inhibition than the marketed drug **Iressa (8).** [175]

Kitano *et al*. have explored non-anilinoquinazolines in search for EGFR TK inhibitors wherein 4-(4-phenylbut-1-yn/en-yl)quinazolines were identified as new and potential scaffolds. 1-Ethynyl-2-phenylaryl/heteroaryl group was found to be a novel substructure at the 4-position of quinazolines. The IC_{50} values of these compounds were in the nanomolar range and the most potent compound was **50** which showed IC_{50} value of 1.8 nM on isolated EGFR TK. These chemotypes represent the first illustration of carbonsubstituted quinazolines at the 4-position with potent inhibitory activity and serve as new starting points for a structurally diverse class of EGFR TK inhibitors. [176]



(50)

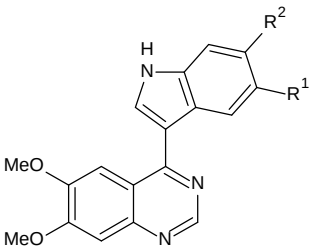


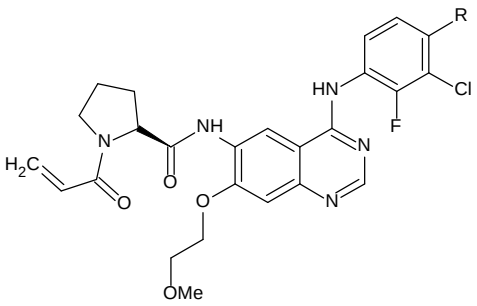
(51)

Two series of new 6-alkoxy-4-substituted aminoquinazolines and their bioisosteric quinoline congeners were designed and synthesized by Abouzid *et al*. Virtual screening was carried out through docking the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) to predict whether these compounds have analogous binding mode to the EGFR inhibitors. The synthesized compounds were tested *in vitro* on human breast carcinoma cell line (MCF-7) in which EGFR was highly expressed. Most of the tested compounds exhibited potent antitumor activity with IC_{50} values in the nanomolar range in particular compound **(51)** displaying the highest activity among the tested compounds with IC_{50} equal to 0.13 nM. [177]

Luth and Lowe have reported a series of 4-(indol-3-yl)quinazolines as highly potent EGFR-tyrosine kinase inhibitors with excellent cytotoxic properties in different cell lines (GI_{50} values between 10^{-6} and 10^{-8} M). Compound **(52)** was the most potent EGFR kinase inhibitor of the series with an IC_{50} of 131 nM. Furthermore, some compounds showed tendencies to inhibit the HER-2 TK too; representative compound **(52A)** inhibited the HER-2

tyrosine kinase at a concentration of 100 nM with a rate of 3% and has IC_{50} of 209 nM, against isolated EGFR TK. [178]





(52) R^1= Br, R^2= H
(52A) R^1= Cl, R^2= F

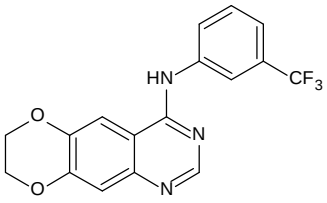
(53) R= H
(53A) R= F

A novel series of (*S*)-1-acryloyl-*N*-[4-(arylamino)-7-(alkoxy)quinazolin-6-yl]pyrrolidine-2-carboxamides were synthesized and evaluated as Her-1/Her-2 dual inhibitors by Cha *et al*. In contrast to the Her-1 selective inhibitors, these novel compounds are irreversible inhibitors of Her-1 and Her-2 tyrosine kinases with the potential to overcome clinically relevant, mutation-induced drug resistance. The selected compounds **(53** and **53A)** showed excellent EGFR inhibition activity even toward the T790M mutation of Her-1 tyrosine kinase (IC_{50} of 24 and 11 nM respectively) with excellent selectivity. The excellent pharmacokinetic profiles of these compounds in rats and their robust *in vivo* efficacy in an A431 xenograft model clearly demonstrate that they merit further investigation as novel therapeutic agents for EGFR-targeting treatment of solid tumors, especially Her-1 selective inhibitor-resistant non-small cell lung cancer. [179]

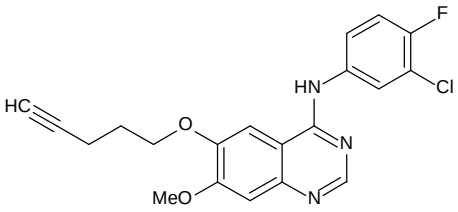
Chilin *et al*. have reported three classes of fused tricyclic (dioxolane, dioxane, and dioxepine) quinazolines as EGFR inhibitors. The cyctotoxic activity of all the compounds was assessed against two cell lines overexpressing and not expressing EGFR, respectively. Most derivatives were able to counteract EGF-induced EGFR phosphorylation and showed better or at least comparable potency with respect to **PD153035**, used as a reference compound. The size of the fused dioxygenated ring was crucial for the cytotoxic activity and for the biological profile. In particular, the dioxane derivatives showed an interesting profile; among these, the most promising derivative was the one bearing the 3′-trifluoromethylaniline substituent **(54)** due to its preferential binding to the inactive form of EGFR. [180]

Ban *et al*. synthesized a series of 4-anilinoquinazolines with C–C multiple bond substituents at the 6-position and investigated their potential to inhibit epidermal growth

factor receptor (EGFR) tyrosine kinase activity. Among the compounds synthesized, alkyne **(55)** (IC_{50} 2.53 ± 0.58 nM**)**, alkenes **(56)** (IC_{50} 3.16 ± 1.33 nM) and **(57)** (IC_{50} 4.45 ± 2.15 nM) significantly inhibited EGFR tyrosine kinase activity. These compounds inhibited EGF-mediated phosphorylation of EGFR in A431 cells, resulting in cell-cycle arrest and apoptosis induction. The C–C multiple bonds substituted at the C-6 position of the anilinoquinazoline framework were essential for the significant inhibitory activity. Compounds with long carbon chains displayed prolonged inhibitory activity. [181]



(54)



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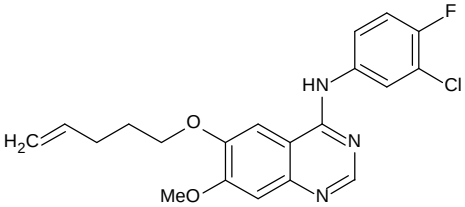

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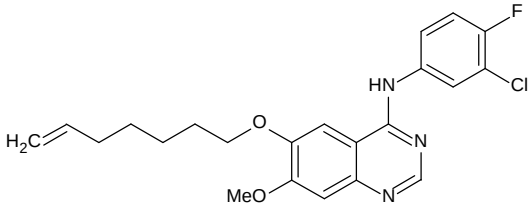

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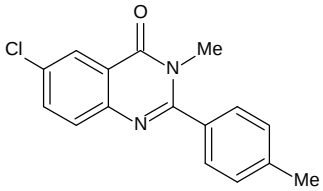

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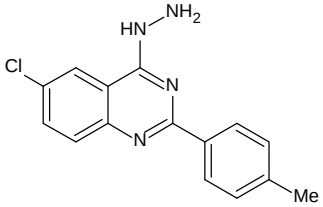
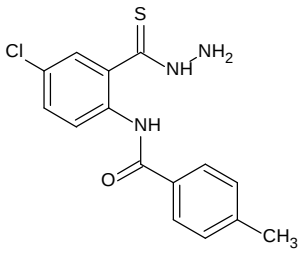
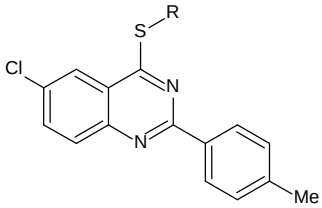


(59)



(60)

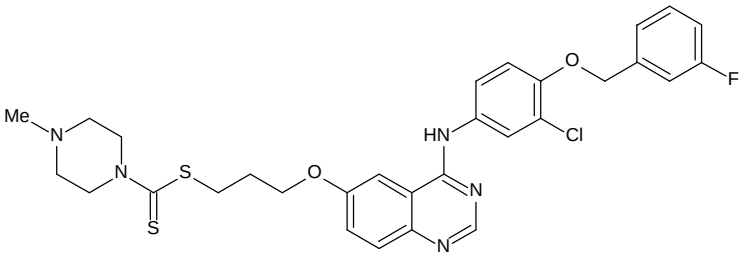


(61) R=CH_2CN **(61A)** R=3-Nitro-2-pyridine

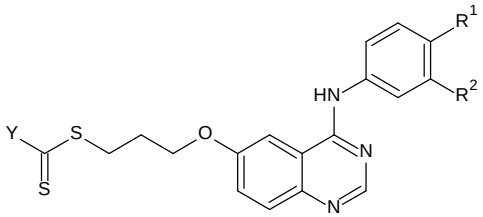
El-Azab *et al*. synthesized novel derivatives of quinazoline and tested them for their antitumor activity against three tumor cell lines. All tested compounds showed potent and selective activity against breast cancer (MCF-7) with IC_{50} range of 3.35-6.81 µg/ml. With regard to broad-spectrum activity compounds **(58-61A)** exhibited potent antitumor activity

against human liver cell line (HEPG2), human breast cell line (MCF-7) and human cervix cell line (HELA) with an IC_{50} range of 3.35-5.59 µg/ml. Molecular docking studies further supported the strong inhibitory activity of **61** and **61A**.[182]

Dithiocarbamic acid esters, a common class of organic molecules, have also attracted great attention due to their cancer chemopreventive and anticancer actions.[184,185] It was reported that when a suitable molecular scaffold was incorporated into dithiocarbamic acid esters as a key pharmacophore, the molecule showed significant anticancer activity, such as dithiocarbamate **(62).**[186] Li *et al*. have designed and synthesized a novel series of EGFR inhibitors by combination of dithiocarbamic acid esters and 4-anilinoquinazolines. The effect of the synthesized compounds on cell proliferation was evaluated by MTT assay in three human cancer cell lines: MDA-MB-468, SK-BR-3 and HCT-116. Two compounds (**63** and **63A**) were found more potent against all the three cells than **34 (Lapatinib)**. SAR studies revealed that the substituents on C6 and C7 positions of quinazoline, the amine component of dithiocarbamate moiety and the linker greatly affected the activity.[186]



(62)



(63) R^1 = OCH_2-C_6H_4-3F R^2= Cl, Y = 1-Methylpiperazine

(63A) R^1 =H, R^2= Br, Y = 1-Methylpiperazine

(63B) R^1 = 3-F-$C_6H_4CH_2O$ R^2 = Cl, Y = Piperazine