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Re: Expediente No. 06-112.530

Solicitud de patente de invención titulada: "DERIVADOS DE PIRIMIDINA PARA TRATAR EL CRECIMIENTO CELULAR ANÓMALO".

Solicitante: PFIZER PRODUCTS INC.

Nuestra Ref.: P2006/000220

RESPUESTA AL OFICIO No. 08813, NOTIFICADO POR FIJACIÓN EN LISTA No. 242,
DE FECHA 09 DE JULIO 2010
(REQUERIMIENTO ARTÍCULO 46)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 46 de la Decisión 486:

1. Presento copia completa del siguiente documento exigido por el Examinador en el examen de búsqueda de anterioridades, según lo previsto en el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina:

1)

C. T. SUPURAN; A. SCOZZAFAVA: "Protein kinase inhibitors as anticancer agents" EXPERT OPIN. THER. PATENTS, vol. 14, no. 1, January 2004 (2004-01), pages 35-53, XP002336074

Atentamente,



CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295

Anexo: Artículo mencionado.

Expert Opinion

1. Introduction
2. New protein tyrosine kinase inhibitors in the patent literature
3. Expert opinion

Protein tyrosine kinase inhibitors as anticancer agents

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There are > 700 protein kinases and 100 protein phosphatases encoded within the human genome. By catalysing protein phosphorylation and dephosphorylation, they play a pivotal role in intracellular signalling and in the regulation of signal transduction pathways. Activation of oncogenes coding for such proteins can lead to the production of kinases that are continually active in the absence of a normal stimulus, leading to increased cell proliferation and/or decreased apoptosis. Receptor and non-receptor protein tyrosine kinases (PTKs) are essential enzymes in cellular signalling processes that regulate cell growth, differentiation, migration and metabolism. Their inhibition by specific inhibitors was recently shown to constitute a new modality for cancer treatment. A patent literature review comprising the years 2000 – 2003 is presented here, as the major drug houses are currently involved in the design and development of novel types of such compounds with applications as antitumour agents.

Keywords: drug design, epidermal growth factor (EGF), Imatinib, inhibitor, tyrosine kinase, vascular endothelial growth factor (VEGF)

Expert Opin. Ther. Patents (2004) 14(1):35-53

1. Introduction

The control of cellular processes, such as cell growth, division and death, involves signal transduction, which commonly involves the transfer of the terminal phosphate moiety of adenosine triphosphate (ATP) to tyrosine residues on substrate proteins, assisted by the tyrosine kinase (TK) family of enzymes [1-9]. Protein phosphorylation and dephosphorylation play a pivotal role in intracellular signalling. There are ~ 700 protein kinases and 100 protein phosphatases involved in the regulation of such signal transduction pathways, encoded within the human genome [1-9]. Activation of oncogenes coding for such proteins can lead to the production of kinases that are continually active in the absence of a normal stimulus, leading to increased cell proliferation and/or decreased apoptosis [1-9]. A major focus of cancer research in recent years has been to identify oncogenic molecules, and the signal transduction pathways in which they are involved, in order to develop specifically targeted drugs. In cancer, as well as in other proliferative diseases, unregulated cell proliferation, differentiation and survival frequently results from abnormal protein phosphorylation. Receptor and non-receptor protein tyrosine kinases (PTKs) are essential enzymes in cellular signalling processes that regulate cell growth, differentiation, migration and metabolism [1-9]. The kinase activity of PTKs is tightly controlled through steric, autoregulatory mechanisms, as well as by the action of protein tyrosine phosphatases. Recent structural studies have revealed several modes of autoregulation governing the catalytic state of these enzymes. Aberrant catalytic activity of many PTKs, via mutation or overexpression, plays an important role in numerous pathological conditions, the most important of which is cancer [1-9]. A schematic representation of the receptor (R)PTKs involved in tumour growth is shown in Figure 1.

Various TK receptors have been identified as regulators of tumour, or tumour vessel, growth [1-12]. Having shown their expression characteristics in different tumour entities, specific inhibitors of the ATP binding sites of these receptors or antibodies were

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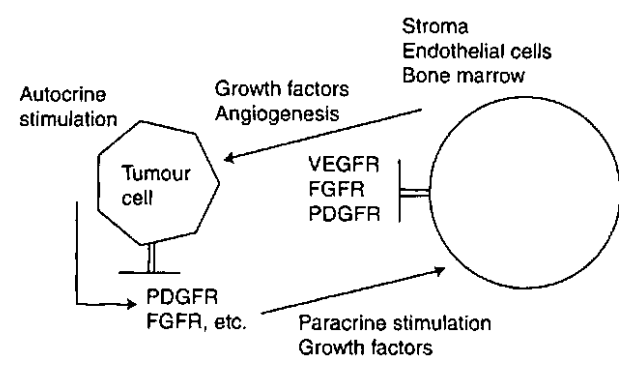


Figure 1. RPTKs involved in tumour growth. This phenomenon is dependent on growth factors derived from the tumour (autocrine stimulation) or the microenvironment (paracrine stimulation). PDGF and FGF are secreted from tumour cells and neighbouring stromal and normal tissue, leading to enhanced growth and survival. VEGF, FGF and PDGF stimulate the branching, extension and survival of endothelial cells, leading to neoangiogenesis [1,2].

FGF: Fibroblast growth factor; FGFR: Fibroblast growth factor receptor; PDGF: Platelet-derived growth factor; PDGFR: Platelet-derived growth factor receptor; RPTK: Receptor protein tyrosine kinase; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.

developed and entered clinical trials or clinical use. The pathogenic role of the TK defines the method of action of the inhibiting drug, whereas the amount of expression in a tumour tissue defines the rationale to use the inhibitor to treat a specific protein. Indications for such drugs will in the future be defined by tumour kinase profiles instead of tumour entities. Thus, the following TKs are currently being targeted by various compounds/drugs in the treatment of cancer: platelet-derived growth factor (PDGF) receptors (PDGFRs), the Abelson (ABL) protein and the KIT protein (also known as stem cell factor [SCF] receptor), the protein kinase A1 (PKA1) and bcl-2/bcl-xL the FLT3 (fms-related TK/Fik2/Stk-2), a receptor tyrosine kinase (RTK) primarily expressed on haematopoietic cells; the epidermal growth factor receptor (EGFR) and ErbB-2 transmembrane TKs [1-12]. The inhibition of angiogenesis through vascular endothelial growth factor (VEGF) receptor (VEGFR) targeting is a strategy that is relatively tumour-selective [1-11]. The EGFR is a transmembrane receptor involved in the regulation of a complex array of essential biological processes, such as cell proliferation and survival. Dysregulation of the EGFR signalling network has been frequently reported in multiple human cancers and has been associated with the processes of tumour development, growth, proliferation, metastasis and angiogenesis. Aberrant expression or activity of EGFR or the closely related p185(erbB2) can promote cell proliferation and survival and thereby contribute to tumourigenesis [1-9]. Antiangiogenic effects have been demonstrated by inhibition of PDGF-, VEGF- and basic fibroblast growth factor (bFGF)-induced angiogenesis *in vivo* and by inhibition of angiogenesis and tumour growth in an experimental bone metastasis model [1-15].

The Met RTK has been shown to be overexpressed or mutated in a variety of solid tumours and has, therefore, been identified as a good candidate for molecularly targeted therapy. Activation of the Met TK by the TPR gene was originally described *in vitro* through carcinogen-induced rearrangement [10]. The TPR-Met fusion protein contains constitutively elevated Met TK activity and constitutes an ideal model to study the transforming activity of the Met kinase. When introduced into an IL-3-dependent cell line it has been found that TPR-Met induces factor independence and constitutive tyrosine phosphorylation of several cellular proteins. One major tyrosine phosphorylated protein was identified as the TPR-Met oncoprotein itself [10].

The PDGFs are a pleiotropic family of peptide growth factors that signal through cell surface, TK receptors (PDGFRs) and stimulate various cellular functions including growth, proliferation and differentiation [11]. To date, PDGF expression has been demonstrated in a number of different solid tumours, from glioblastoma to prostate carcinomas. In these various tumour types, the biological role of PDGF signalling can vary from autocrine stimulation of cancer cell growth to more subtle paracrine interactions involving adjacent stroma and vasculature [11]. The first TK inhibitor to be used clinically, imatinib mesylate (compound 1, formerly STI-571, Gleevec®, Novartis Pharm. Corp.) blocks activity of the Bcr-Abl oncoprotein and the cell surface TK receptor c-Kit and as such was recently approved for several indications in the treatment of chronic myeloid leukaemia and gastrointestinal stromal tumours [1-9,11-15]. In both of these examples the target protein was identified by an oncogenic, activating mutation. Imatinib mesylate is also a potent inhibitor of PDGFR kinase and is currently being evaluated for the treatment of chronic myelomonocytic leukaemia and glioblastoma multiforme, based upon evidence in these diseases of activating mutations in PDGFR [1-9,11-14]. The molecular pathogenesis of chronic myelogenous leukaemia (CML) in particular, depends on formation of the Bcr-Abl oncogene, leading to constitutive expression of the TK fusion protein, Bcr-Abl. Based on these observations, imatinib was developed as a specific inhibitor for the Bcr-Abl PTK. Other PTK inhibitors (PTKIs) in advanced clinical trials include gefitinib (Iressa®, AstraZeneca, compound 2), inhibiting the EGF-RTK; trastuzumab (Herceptin®, Genetech; a monoclonal antibody), inhibiting the Her2/neu TK; PTK-787/ZK-222584 (vetalanib, Novartis AG; compound 3), inhibiting the VEGF-RTK, whereas ZD-6474 (AstraZeneca, compound 4) and SU-6668 (Sugen, Inc., compound 5) are representatives of multitarget TK inhibitors.

In non-small cell lung cancer many investigators have focused their attention on the EGFR because this membrane protein, which has an extracellular ligand binding domain as well as TK activity on the intracellular portion of the molecule, is expressed in a relatively high proportion of non-small cell lung cancers. Gefitinib, which was the first EGFR-specific TK inhibitor to be extensively tested in non-small cell lung cancer, has shown single agent activity in non-small cell lung cancer. Subsequently,

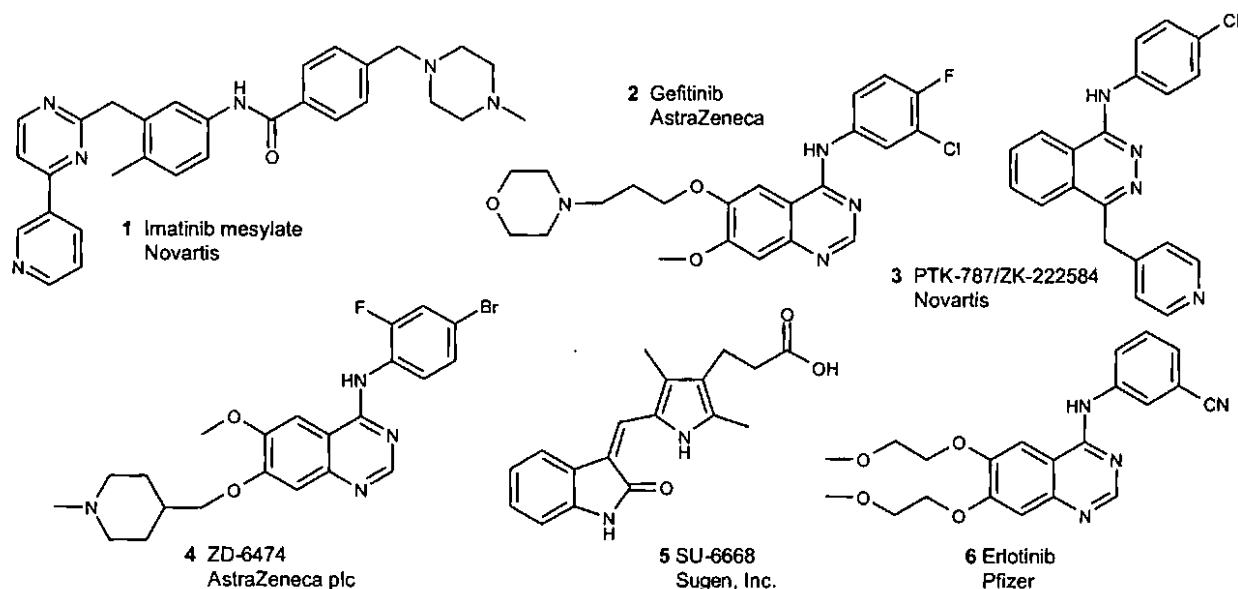


Figure 2. Protein tyrosine kinase inhibitors.

erlotinib (Tarceva[®], Pfizer, Inc.; compound 6), another EGFR-specific tyrosine inhibitor, has also demonstrated single agent activity in non-small cell lung cancer. Phase III trials of erlotinib (compound 6), alone or in combination with chemotherapy, have been completed and data are being analysed. Several dual inhibitors of erbB1 and erbB2 (PKI-166, GW-572016, EKB-0569) have been, or are being, tested in Phase I trials. In addition, CI-1033, a pan-erb inhibitor, is also being tested in Phase I studies. Although the erb TK inhibitors are attractive agents to use in treating non-small cell lung cancer because of their relatively benign toxicity profile, more data are needed to define the role of these agents in non-small cell lung cancer [16].

RTK activation contributes to cell viability during cytotoxic therapy. The novel broad spectrum RTK inhibitor, SU-11248, inhibits VEGFR2, PDGF receptor, c-kit and fetal liver TK3 [14].

The ongoing clinical trials of different PTKIs, together with their protein target(s) and indications are shown in Table 1. As seen from these data, this is an emerging field for the design of novel anticancer therapeutics, with many new discoveries reported continuously [16-23]. This review will concentrate on the new reports of different PTKIs in the patent literature in the period 2000 – 2003, for which there is an abundance of new data in this field and no recent review is available. Previous valuable reviews regarding the development of the clinically used inhibitors as well as the first research that led to them are available [1-3,18].

2. New protein tyrosine kinase inhibitors in the patent literature

Due to the very large number of patents claiming novel types of PTKIs with antitumour properties, as well as the heterogeneous chemical nature of these derivatives, they will be

discussed in the following sections, considering the drug houses that discovered them (Figures 3 – 12).

2.1 Novartis

After developing the first PTKI to reach clinical use, imatinib (compound 1), Novartis have proceeded to discover many other different classes of such derivatives in the last 3 years. Thus, N-aryl(thio)anthranilic acid amides of type 7 (Figure 3) and their inhibition of VEGF-RTK, useful in the treatment of neoplastic disease, were claimed. These compounds were stated to be useful in inhibiting angiogenesis and for treating inflammatory rheumatoid diseases (e.g., rheumatoid arthritis) and pain. The *in vitro* activities of three compounds against Flt-1 VEGF-RTK were presented, some compounds showing median inhibitory concentration (IC₅₀) values in the low micromolar range [101]. Novel 2-amino-6-anilinopurine derivatives of type 8 and their use for the chemotherapy of tumours or osteoporosis, were then claimed [102]. The compounds (8) were stated to inhibit p34cdc2/cyclin Bdc13 kinase and PTK pp60c-src and to be useful for treatment of breast cancer, bowel cancer, bone metastases, psoriasis, Paget's disease and hypercalcaemia. These compounds showed IC₅₀ values of 0.05 – 20 nM but no specific data were presented. *In vivo*, the activity of the compounds (8) against tumours transplanted subcutaneously into female BALB/c nude mice was investigated and they were stated to have produced a significant reduction in the average tumour volume compared to the untreated control animals but no data were presented [102]. Novel arylaminoisoquinoline derivatives of type 9 and their use for treating diseases sensitive to inhibition of angiogenesis or VEGF-RTK, were also claimed [103]. These compounds are stated to inhibit VEGF-dependent cell proliferation and to be useful in the treatment of many angiogenic disorders including severe asthma, fibrotic disorders, neoplasm, diabetic retinopathy

5

Table 1. Clinical trials of different protein tyrosine kinase inhibitors, with their protein target(s) and indications [7].

PTK target	Compound	Company	Tumour	Phase
Bcr-Abl	Gleevec® (imatinib mesylate)	Novartis	Chronic myelogenous leukemia	I
			Childhood acute lymphatic leukemia	I
			Childhood acute myelogenous leukemia	I
c-KIT	Gleevec	Novartis	GIST	II/III
			Melanoma; ovarian cancer; small cell lung cancer	II
			Childhood soft tissue sarcoma	II
PDGF-R	Gleevec	Novartis	Malignant glioma	I/II
			Childhood brain glioma	I/II
			Urethral cancer, bladder cancer	II
EGFR/HER-1	Iressa® (gefitinib)	AstraZeneca	Non-small cell lung cancer	I/II
			Adult glioblastoma multiforme	II
			Ovarian, head and neck, prostate	II
			Endometrial cancer	II
			Colon	I
VEGF-R	PTK787/ZK222584	Novartis	Various tumours	I
Multiple PTKs ZD6474 (VEGFR, EGFR, PDGFR)	ZD-6474	AstraZeneca	Breast cancer	II
			Various cancers	I
Multiple PTKs Su6668 (VEGFR, EGFR, PDGFR)	SU-6668	Sugen	Various cancers	I

Abl: Abelson protein; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; GIST: Gastrointestinal stromal tumour; HER: Human EGF receptor; PDGFR: Platelet-derived growth factor receptor; PTK: Protein tyrosine kinase; VEGFR: Vascular endothelial growth factor receptor.

and age-related macular degeneration. Five compounds were tested as inhibitors of Flt-1 VEGF-RTK and showed IC₅₀ values between 344 and 802 nM [103]. Combination formulations comprising an inhibitor of α -1 acidic glycoprotein and one ABL, PDGFR or Kit RTK inhibitor were also claimed [104]. The use of an inhibitor of this glycoprotein enhances the bioavailability of the TK inhibitor and is suggested to provide a method of overcoming the development of resistance. The method is claimed to be particularly applicable to imatinib (compound 1). Thus, the anticancer activity of imatinib was shown to decrease on KU812 cells when incubated in the presence of increasing concentrations of α -1 acidic glycoprotein. At 2 mg/ml, this decreased the IC₅₀ value from 50 nM to 3 μ M. The binding capacity of this protein for the specified compound was determined as 8.7 l/mol. In mice, increasing tumour load was shown to increase the plasma levels of the glycoprotein from 0.096 to 1.6 mg/ml. Addition of erythromycin, an inhibitor of the glycoprotein, was shown to restore the anticancer potency of the specified compound 1 [104]. Novel 2-aminonicotinamide derivatives (10) and their use for the treatment of disorders requiring inhibition of VEGF-RTK activity, were claimed [105]. These compounds were disclosed to be useful for inhibiting angiogenesis, for the treatment of pain and for preventing the metastatic spread of tumours and the growth of micrometastases. The compounds were evaluated for *in vitro* activity against Flt-1 VEGF-RTK and the IC₅₀ values were stated to be in the range 1 – 100 nM but no

specific biological data were presented. Two further *in vivo* assays were described to evaluate the compounds for antitumour and antinociception activity [105]. Novel anthranil-alkyl or cycloalkyl amides of type 11 and their use for treating tumours, psoriasis, arthritis, haemangioma, angiofibroma, a range of ocular, renal and fibrotic diseases, nerve injury, restenosis, atherosclerosis and VEGF-induced oedema, were claimed [106]. These compounds were disclosed to be angiogenesis inhibitors and were claimed to inhibit the TKs KDR and FLT, which are part of the signalling pathway of VEGF receptors. Six compounds inhibited TK with IC₅₀ values of 39 – 1000 nM (with 80 nM for the specified compound) against KDR TK [106]. Novel substituted aza- or polyanthranilic acid amides related to compound 10 and their use for treating diseases sensitive to inhibition of angiogenesis were claimed [107]. These compounds were disclosed to be inhibitors of the same PTKs mentioned above [107]. Novel arylaminopurine derivatives of type 12 and their use for the treatment of inflammatory or obstructive airway disease are claimed. The compounds are disclosed as inhibitors of syk PTK and inhibitors of eosinophil activation. These compounds are disclosed to be useful for the treatment of asthma, acute respiratory distress syndrome (ARDS), bronchitis, emphysema, pneumoconiosis, pneumonia, skin allergies, tumours and ophthalmic inflammation [108]. The novel use of imatinib (compound 1) for the treatment of gastrointestinal stromal tumours (GIST), in particular as the monomethanesulfonate salt at a dose of 200 – 600mg once a

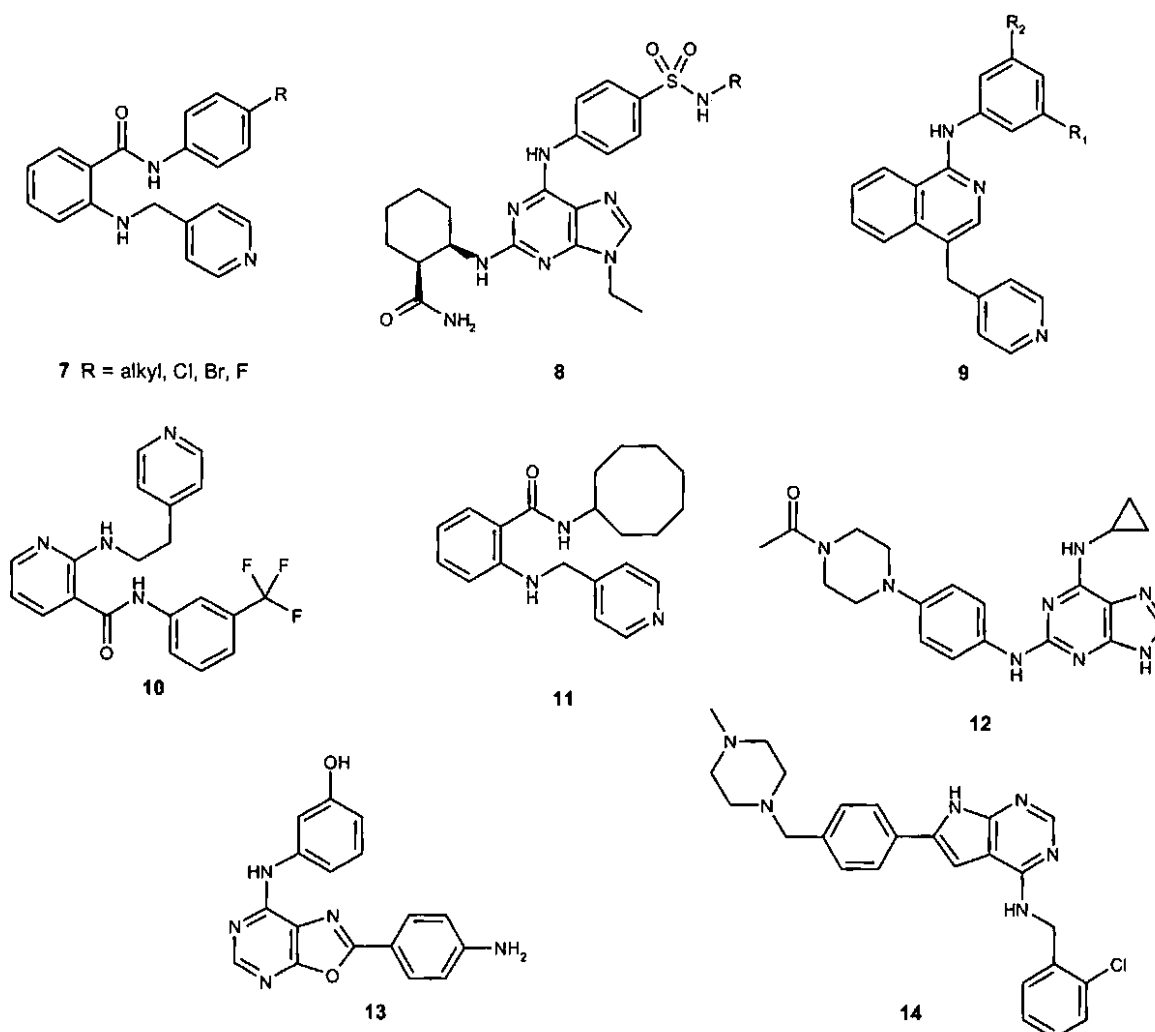


Figure 3. Protein tyrosine kinase inhibitors from Novartis.

day for at least 3 months, was claimed [109]. The results of a Phase II clinical study in 147 patients with unresectable and/or metastatic malignant GIST were also presented. The patients received the specified compound 400 or 600 mg orally, once a day for up to 24 months. A partial response was confirmed in 59 patients and 119 patients showed a decrease in tumour size of > 25% on at least one assessment [109]. Combinations of an epothilone derivative and either a PDGF-RTK inhibitor or an EGF inhibitor and their use for the treatment of proliferative diseases, especially solid tumours were also claimed [110]. Preferred PDGFR TK inhibitors were 2-anilinopyrimidine derivatives (of the imatinib type) and the preferred EGF inhibitor was PKI-166. The specified combination was compared with the individual components in mice bearing C6 glioma or KAT-4 thyroid carcinoma xenografts. The evaluation of various dose combinations showed some evidence of synergy in reducing the volumes of C6 tumours. The PDGF inhibitor, at 100 mg/kg orally, once a day, was ineffective against the KAT-4 tumours but

potentiated the reduction in tumour size achieved with EPO-906 at 0.3 mg/kg (s.c.) once a week, from 31 to 55% [110]. Novel oxazolo and/or furopyrimidine derivatives (13) and their use in the treatment of disorders associated with deregulated angiogenesis and/or for the treatment of benign or malignant tumours were claimed [111]. The compounds were also stated to be useful for the treatment of rheumatoid diseases, pain, psoriasis, carcinomas, neurodegenerative diseases, renal diseases, diabetic retinopathy, autoimmune diseases, inflammation, fibrotic diseases, diabetes mellitus, endometriosis, asthma, atherosclerosis and restenosis. The compounds were disclosed to be TK inhibitors [111]. Novel 4-amino-6-phenyl-pyrrolo[2,3-d]pyrimidine compounds of type 14 and their use as PTKIs were claimed [112]. The compounds were stated to be useful for the treatment of proliferative diseases such as benign and malignant tumours, metastasis and psoriasis. The compounds were disclosed to be particularly active against the EGF receptor (EGFR) and ErbB-2 kinase. The IC_{50} values ranged from 0.0018 to 0.1387 μ M [112].

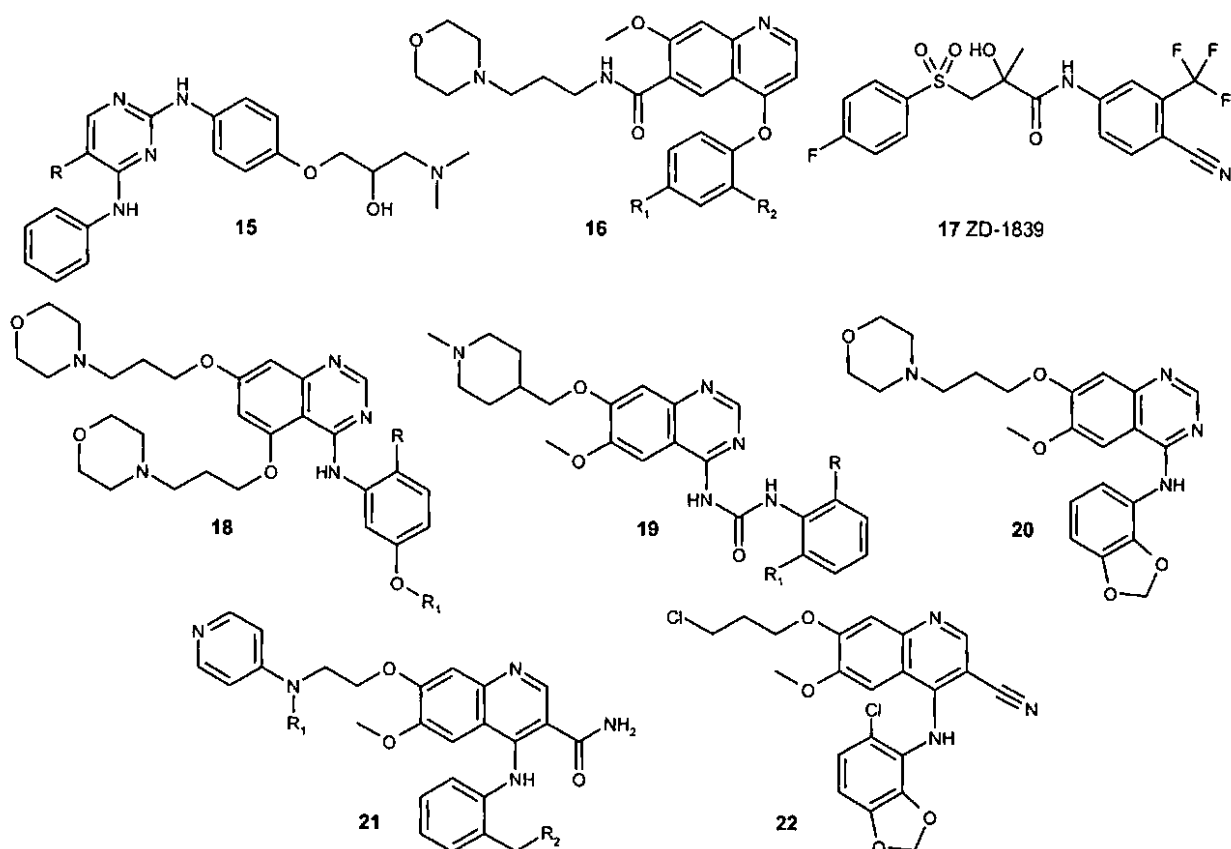


Figure 4. Protein tyrosine kinase inhibitors from AstraZeneca.

2.2 AstraZeneca

AstraZeneca also has an intensive programme for the development of PTKs with potential use as antitumour agents. Novel pyrimidine derivatives of type 15 (Figure 4), useful for treating cancer, were claimed [113]. They were disclosed as inhibiting the PTK focal adhesion kinase (FAK). They are useful for treating solid tumours, leukaemia, fibroproliferative and differentiative disorders, acute and chronic inflammation, bone diseases, ocular diseases, autoimmune diseases, restenosis, nephropathies and psoriasis. TK inhibitory activity using human FAK was determined and the IC_{50} values for two compounds were 0.032 and 0.07 μ M [113]. Novel quinoline derivatives structurally related to gefitinib (compound 2), of type 16 and their use in the prevention or treatment of T-cell-mediated diseases were claimed [114]. The compounds are stated to be selective inhibitors of p56lck TK and of potential use as immunoregulative or immunosuppressive agents in the treatment of immune diseases such as transplant rejection and rheumatoid arthritis [114]. Novel combinations of EGFR TK inhibitors and non-steroidal antiandrogens, administered simultaneously, sequentially or separately, for use in the treatment of prostate cancer were claimed. In particular, combinations of the EGFR inhibitor ZD-1839 (compound 17) are claimed. No biological data are presented although it is stated that the specified combination produces a substantial reduction in

tumour proliferation in murine xenograft models bearing CWR-22 or PC-3 tumours [115]. Novel quinazoline derivatives of type 18, a process for their preparation and their use in the treatment of solid tumour diseases, were also claimed. It is stated that the compounds are also useful for treating inflammatory diseases, fibrotic diseases, glomerulonephritis, multiple sclerosis, psoriasis, skin hypersensitivity, blood vessel disease, allergic asthma, insulin-dependent diabetes, diabetic retinopathy and diabetic nephropathy and that they act as Src non-RTK inhibitors, particularly c-Src and c-Yes, whilst demonstrating little activity against EGF- and VEGF-RTK. *In vitro* enzyme, fibroblast proliferation and microdroplet migration assays and an *in vivo* A549 xenograft growth assay were described but no specific biological data were presented [116]. Novel quinazoline derivatives (19), their pharmaceutically acceptable salts and their use for producing an antiangiogenic and/or vascular permeability-reducing effect, were claimed. The compounds are also claimed to be inhibitors of the VEGF-RTK and are disclosed to be potentially useful for the treatment of cancer, particularly primary and recurrent solid tumours which depend significantly on VEGF for their growth, such as certain tumours of the colon, breast, prostate, lungs, vulva and skin and also diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, excessive

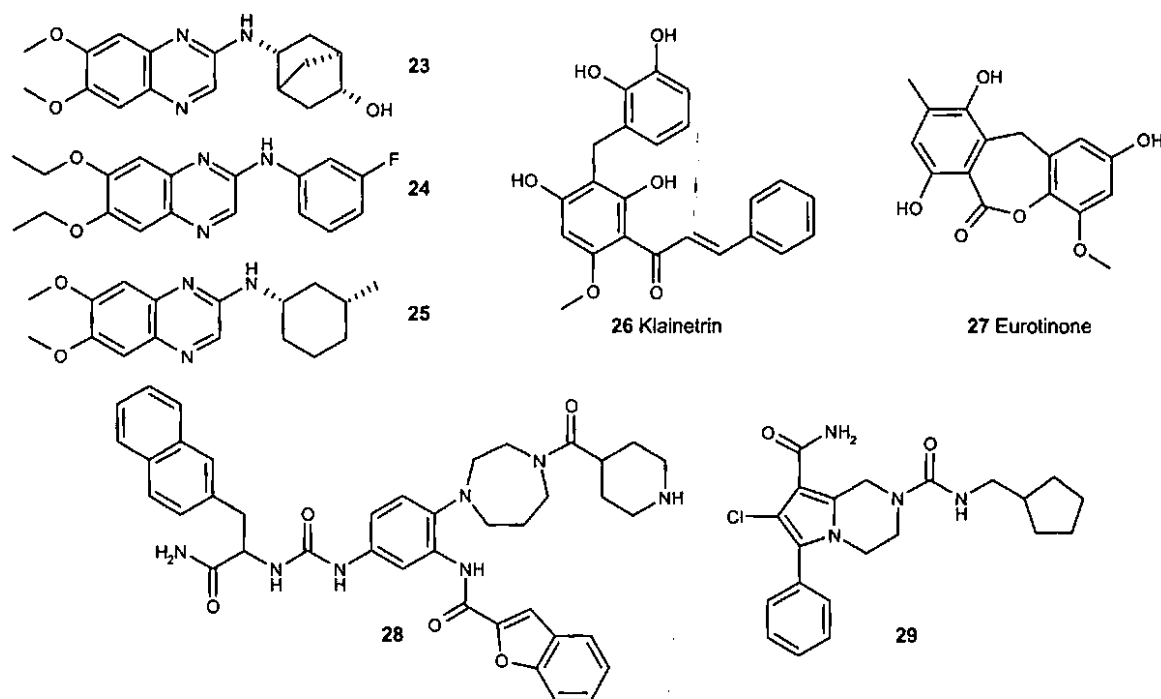


Figure 5. Protein tyrosine kinase inhibitors from Aventis.

scar formation and adhesions, endometriosis, dysfunctional uterine bleeding and ocular disease with retinal vessel proliferation [117-119]. Novel quinazoline derivatives (20), structurally related to compounds 18 and 19, discussed previously, and their use in the treatment of solid tumours, were claimed. The compounds are disclosed to be inhibitors of the Src family of non-RTKs but no resultant biological data are presented [120]. A large series of quinoline derivatives (21 and 22) and their use in the treatment of solid tumour disease, were claimed. The compounds are stated to be selective inhibitors of the non-RTKs such as c-Src or c-Yes over other TKs and to be useful for treating inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, fibrotic diseases such as hepatic cirrhosis, glomerulonephritis, multiple sclerosis, psoriasis, insulin-dependent diabetes, diabetic retinopathy and nephropathy and blood vessel diseases such as atherosclerosis and restenosis [121-125].

2.3 Aventis

Novel substituted quinoline and quinoxaline derivatives of types 23 – 25 (Figure 5), similar to erlotinib, and methods of their use for the treatment of hyperproliferative disorders, atherosclerosis, inflammation, restenosis, brain cancer, ovarian cancer, lung cancer, colon cancer, prostate cancer, Kaposi's sarcoma and malignant melanoma, were claimed. Methods of using these compounds to inhibit PTKs, particularly the PDGF and Lck kinases, were further claimed [126-128]. Klainerin (compound 26) was isolated from the plant *Uvaria klaineri* and is claimed to be useful as a cytostatic agent. The compounds

related to compound 26 are also claimed to act as cyclin-dependent kinase (CDK) and TK inhibitors and are stated to be useful for treating tumours. The specified compound, klainerin (26), was the most potent, with IC_{50} values of 6.48, 6.04 and 1.14 μ M versus TK, CDK2 and CDK4, respectively [129]. Eurotinone (compound 27) and its O-alkyl derivatives, a process for their fermentative preparation using the novel fungus *Eurotium echinalutum* Delacroix and their use as cytostatic and angiogenesis-inhibiting agents were also claimed by the same group [130]. These compounds are claimed to act as kdr kinase inhibitors and to be useful for treating malignant diseases [130]. Novel substituted amide, sulfonamide and urea derivatives of type 28, were claimed to act as protein kinase inhibitors and to be useful for treating cancer, diabetes, immune disorders, cardiovascular and CNS disorders, renal failure and osteoporosis.

2.3.1 Biology

The ability of the compounds to inhibit c-Src protein kinase was measured *in vitro* [131]. Novel 3,4-dihydro-1H-pyrazolo[1,2-a]pyrazine derivatives (29) and their use for the treatment of cancer, in particular solid tumours, especially breast cancer, colon cancer and lung cancer, were claimed. The use of the compounds as TK inhibitors, especially of FAK and IGFR1 is also claimed. The compounds were further stated to be JNK inhibitors and to be useful in the treatment of inflammation since they can inhibit the release of inflammatory cytokines such as $TNF-\alpha$. A number of kinase and cellular assays and *in vivo* assays were described [132].

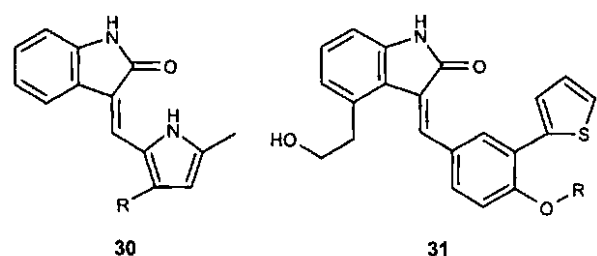


Figure 6. Protein tyrosine kinase inhibitors from Sugen.

2.4 Sugen

A novel method of treating PTK-mediated disorders, such as cancer, diabetes, autoimmune disorders, hyperproliferative disorders, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, inflammatory disorders and angiogenesis, which comprises the administration of 3-heteroarylidenyl-2-indolinone derivatives of type 30 (Figure 6) has been claimed. The specified compound was tested *in vivo* against a variety of tumour cell lines implanted into immunocompromised mice. The summarised results show that, at 12.5 – 25 mg/kg/day, one of the investigated compounds (30, R = CH₃) demonstrated 30 – 80% inhibition against a broad spectrum of tumours, including those of which the growth is driven by PDGF, EGF and Her2 [133]. Structurally related indoline derivatives of type 31, pharmaceutical compositions comprising them as the active ingredient and methods of their use as protein kinase and cellular tyrosine and serine-threonine kinase inhibitors for the treatment of cancer, immunological, hyperproliferative, cardiovascular and inflammatory disorders, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, atherosclerosis, diabetes and angiogenesis, were then claimed. The human EGF receptor (HER)2 inhibitory activity of the compounds was investigated *in vitro*. The tabulated results show that the specified compounds inhibited HER2 with an IC₅₀ value of 8.5 μM [134].

2.5 Merck

Compounds of types 32 – 34 (Figure 7), acting as TK inhibitors useful for treating cancer of the brain, genitourinary tract, lymphatic system, stomach, larynx, pancreas, breast and lung, adenocarcinoma and glioblastomata, were claimed. The compounds were further claimed for use in treating disorders in which neo-angiogenesis is implicated, such as ocular disease, including retinal vascularisation, diabetic retinopathy, age-related macular degeneration, rheumatoid arthritis, psoriasis, contact dermatitis and delayed hypersensitivity reactions. VEGFR kinase and human umbilical vein endothelial cell mitogenesis assays were described and the compounds are stated to exhibit IC₅₀ values of between 0.01 and 5.0 μM but no specific biological data were presented [135–137]. Novel pyridin-2-one and pyrimidin-2-one derivatives (35), pharmaceutical compositions comprising them, optionally including an oestrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxic agent, an antiproliferative agent, a prenyl-protein transferase

inhibitor, an HMG-CoA reductase inhibitor, an HIV protease inhibitor, a reverse transcriptase inhibitor and another angiogenesis inhibitor, and methods of their use for the treatment of cancer, including cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung, histiocytic lymphoma, lung adenocarcinoma, small cell lung cancers, pancreatic cancer, glioblastomata and breast carcinoma, ocular diseases involving angiogenesis, retinal vascularisation, diabetic retinopathy, age-related macular degeneration, inflammatory diseases, such as rheumatoid arthritis, psoriasis, contact dermatitis and delayed hypersensitivity and bone pathologies associated with osteosarcoma, osteoarthritis and rickets, were claimed [138]. The compounds were stated to inhibit VEGF-stimulated mitogenesis of human vascular endothelial cells *in vitro* with IC₅₀ values in the range 0.01 – 5.0 μM but no specific data are presented [138]. Novel heterocyclic compounds of type 36, pharmaceutical compositions comprising them and methods of their use as PTK inhibitors for treating or preventing various cancers, angiogenesis, ocular disease, diabetic retinopathy, age-related macular degeneration, psoriasis, rheumatoid arthritis, contact dermatitis, delayed hypersensitivity reactions, osteosarcoma, osteoarthritis and rickets, were claimed. In an *in vitro* assay, the compounds are stated to have inhibited VEGF-stimulated mitogenesis of human vascular endothelial cells in culture with IC₅₀ values of 0.01 – 5.0 μM but no specific data are presented [139]. Novel substituted benzimidazolyl (37) and indole (38) derivatives, as well as methods for their use in the treatment of TK-dependent or angiogenesis-implicated diseases, including cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx, lung, small cell lung, pancreas or breast, ocular disease, retinal vascularisation, diabetic retinopathy, age-related macular degeneration, inflammatory diseases, rheumatoid arthritis, psoriasis, contact dermatitis, delayed hypersensitivity reactions and bone-associated pathologies, particularly osteoarthritis or rickets and for reducing or preventing tissue damage following a cerebral ischaemic event, were claimed. The compounds are stated to show inhibitory activity *in vitro* in VEGF receptor kinase and human umbilical vein endothelial cell (HUVEC) mitogenesis assays but no resultant data are presented [140,141]. Quinolnonyl pyrrolopyridine derivatives of types 39 and 40 and methods using these compounds and compositions to treat cancer or diseases where angiogenesis or vasculogenesis is implicated or TK-dependent diseases, were claimed. The compounds are said to inhibit, modulate or regulate signal transduction of both receptor type and non-receptor type TKs. Protocols for measuring VEGF (both KDR and flt-1) receptor kinase activity using incorporation of radiolabelled phosphate into pEY substrate caused by the intracellular domains of human KDR or flt-1 expressed in Sf21 insect cells and for measuring the mitogenic response of HUVECs to VEGF using incorporation of [³H]-thymidine into cellular DNA were described but no results were given [142,143]. Novel heterocyclic compounds of type 41, their salts and their use as TK inhibitors for the treatment of cancer, angiogenesis disorders, diabetic retinopathy, inflammation and bone disease were claimed. A number of *in vitro* assays are described. The compounds are stated to have kinase inhibitory

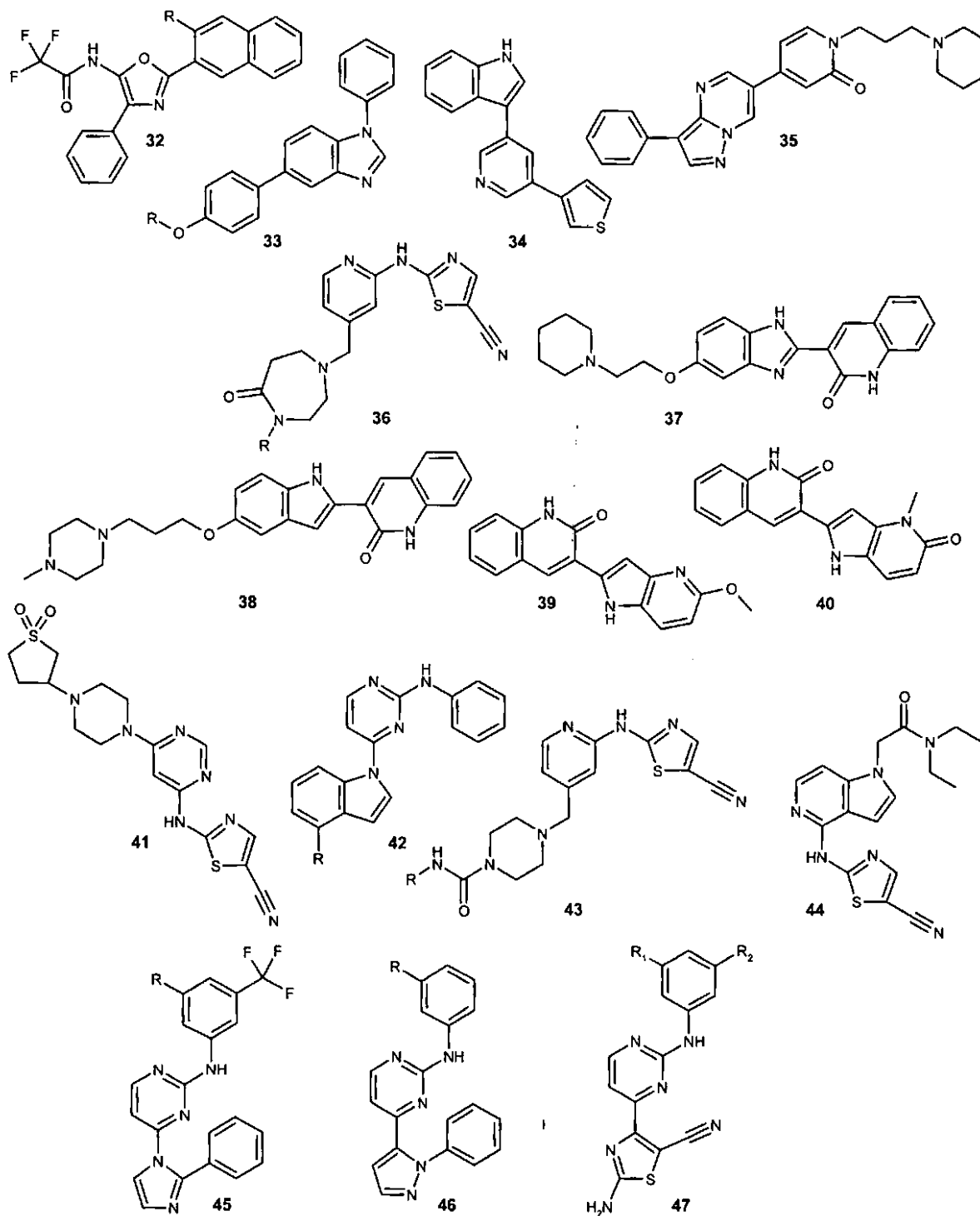


Figure 7. Protein tyrosine kinase inhibitors from Merck.

activity and to inhibit VEGF-stimulated mitogenesis of human vascular endothelial cells with IC_{50} values of 0.01 – 5.0 μ M but no specific data are presented [144]. A method of treating tumours and metastases was claimed through the use of a combination of a TK receptor antagonist and an antiangiogenic agent and

compositions containing them. The combination may be administered with cytotoxic agents or with radiation therapy. Agents, including monoclonal antibodies, which block ErbB receptors, particularly EGF receptors and those which inhibit endothelial cell integrin receptors, are specifically claimed for use in

the combination. The combination is expected to have a synergistic action resulting in a requirement for lower doses of the components and leading to reduced side effects. Kits comprising these combinations are also claimed. An example details the treatment of a patient with squamous cell and then adenosquamous carcinoma of the superior maxilla. Following tumour resection, radiation and chemotherapy, regression and restoration of hearing was observed. After the patient's condition worsened, dosing included EMD-72000 (425 antibody, 200 mg, i.v.), EMD-121974 (Cilengitide, 1200 mg/m²) and gemcitabine. Tumour mass was found to disintegrate and the treatment considered impressive. The patient's condition remained improved [145]. Novel pyrimidine (compound 42) and thienylaminopyridine (43) derivatives and methods of their use for the treatment or prevention of cancer, particularly cancer of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung, histiocytic lymphoma, lung adenocarcinoma, small cell lung cancers, pancreatic cancer, glioblastoma, breast carcinoma, angiogenesis disorders including ocular disease, retinal vascularisation, diabetic retinopathy, age-related macular degeneration, inflammatory diseases, particularly rheumatoid arthritis, psoriasis, contact dermatitis and delayed hypersensitivity, TK-dependent diseases, bone diseases, selected from osteosarcoma, osteoarthritis and rickets and pre-eclampsia were claimed. The use of the compounds to inhibit at least two TK receptors, preferably selected from KDR, EGFR and SRC and their use in combination with other agents were also claimed [146,147]. Novel heterocyclic compounds of types 44 – 47, pharmaceutical compositions containing them and their use for treating TK-dependent diseases, including cancer, in particular histiocytic lymphoma, lung adenocarcinoma, small cell lung cancers, pancreatic cancer, glioblastoma and breast carcinoma, retinal vascularisation, diabetic retinopathy, age-related macular degeneration, inflammatory disease (e.g., rheumatoid arthritis, psoriasis and contact dermatitis), bone disease (e.g., osteosarcoma, osteoarthritis and rickets), pre-eclampsia and tissue damage due to cerebral ischaemia or bacterial meningitis, alone or in combination with another compound, were claimed [148-151].

2.6 GlaxoSmithKline

Substituted quinazoline derivatives of type 48 and their use for the treatment of a disorder mediated by aberrant protein kinase activity, were claimed. These compounds were stated to be PTK inhibitors and to be particularly useful for the treatment of disorders caused by aberrant c-erbB-2 and/or EGF-r and lck and/or zap70 activity, such as breast, ovarian, gastric, pancreatic, non-small cell lung, bladder, head and neck cancers, psoriasis and rheumatoid arthritis.

2.6.1 Biology

The EGF-r and c-erbB-2 PTK inhibitory activities of these compounds were measured *in vitro*. Some compounds inhibited both kinases with IC₅₀ values of < 0.1 µM. This compound (48, Figure 8) also inhibited the growth of HB4a c-erbB-2, BT474, HN5 and N87 tumour cell lines with

IC₅₀ values of < 5 µM [152]. Novel 4-anilinoquinazoline derivatives of type 49, pharmaceutical compositions comprising them as the active ingredient and methods of their use for the treatment of a disorder mediated by aberrant protein kinase, particularly Erb-B2, c-Erb-B4 or EGFR, activity, especially cancer and psoriasis, were also claimed. These compounds are stated to be useful for the treatment of breast, ovarian, gastric, pancreatic, non-small cell lung, bladder or head and neck cancers. The Erb-B4, Erb-B2 and EGFR kinase inhibitory activity of the compounds was investigated. The specified compound inhibited Erb-B4 with an IC₅₀ value of 0.10 – 1.0 µM and the other kinases with IC₅₀ values of < 0.10 µM. In methylene blue growth inhibition assays using human breast (BT474), head and neck (HN5) and gastric tumour (N87) cell lines, the specified compound showed IC₅₀ values of < 0.10, < 0.10 and 0.10 – 1.0 µM, respectively [153]. A novel quinoline derivative (50), its salts and solvates and its use for the treatment of a disorder mediated by aberrant p56lck activity and for the treatment of rheumatoid arthritis were claimed. This compound is disclosed to be a PTK inhibitor [154]. Novel substituted oxindole derivatives 51 and 52 and methods of their use for treating disorders mediated by mitogen-activated kinases, particularly TrkA, such as pain and cancer, were claimed. It is stated that inhibition of TrkA and other Trk kinases may be useful in the treatment of these disorders as they serve as mediators of nerve growth factor (NGF)-driven biological responses [155,156].

As SmithKline Beecham has now been incorporated by Glaxo, the PTKI developed by this drug house will also be discussed here. Thus, novel alkylpiperidylalkylquinoline derivatives of type 53 and their use for the treatment of bacterial infections, are claimed. These compounds are stated to be PTK inhibitors and to be active against Gram-positive and Gram-negative organisms [157]. Imidazole derivatives of type 54 were claimed to be Tie2 receptor inhibitors (TK receptors with immunoglobulin and EGF homology domains) and to be useful for the treatment of angiogenesis disorders, arthritis, diabetic retinopathy, atherosclerosis, carcinoma, metastasis and inflammation [158]. Novel 2-amino-thiophene-3-carboxamide derivatives of type 55 and their use in methods of treating cancer, diseases characterised by cellular proliferation associated with neovascularisation or vascular permeability and disorders mediated by inappropriate angiogenesis or VEGFR2 kinase or TIE-2 kinase activity were claimed. The compounds are stated to be useful for the treatment of blood-vessel proliferative disorders, fibrotic disorders, mesangial cell proliferative disorders, metabolic disorders and chronic wound healing, inflammation, neurodegenerative diseases, diabetic retinopathy, macular degeneration, ocular neovascularisation and haemangiomas. The compounds were stated to be inhibitors of VEGFR2 kinase and/or TIE-2 kinase but no specific data are presented [159].

2.7 Bristol-Myers Squibb

Novel pyrrolotriazine derivatives of type 56 (Figure 9) and their use as angiogenesis inhibitors, protein kinase inhibitors and

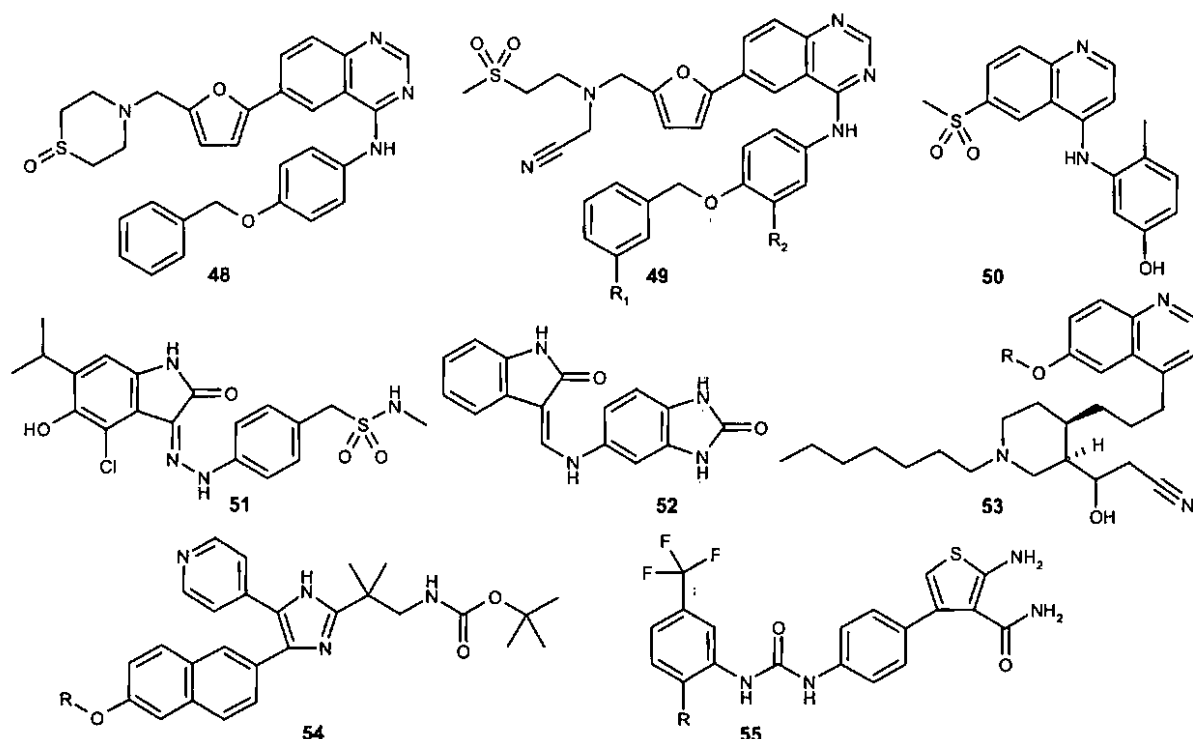


Figure 8. Protein tyrosine kinase inhibitors from GlaxoSmithKline.

TK inhibitors for the treatment of proliferative diseases, cancer, inflammation and autoimmune diseases were claimed. The protein kinase inhibitory activity of the compounds was determined in *in vitro* assays employing VEGFR2, fibroblast growth factor receptor (FGFR), HER1 and HER2 kinases. The compounds are stated to show IC_{50} values of 0.003 – 25 μ M against VEGFR2, FGFR, HER1 and HER2 kinases. No specific data were presented [160]. Novel cyclic amide derivatives of type 57, optionally in combination with an anti-inflammatory, antiproliferative or chemotherapeutic agent, immunosuppressant, anti-cancer agent or PTK inhibitor, were claimed. These compounds were claimed to act as PTK inhibitors for treating transplant rejection, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, lupus erythematosus, graft-versus-host disease, T-cell-mediated hypersensitivity and other T-cell-mediated disorders, psoriasis, Hashimoto's thyroiditis, Guillain-Barre syndrome, cancer, contact dermatitis, allergic disease, asthma, atopic dermatitis, allergic rhinitis, chronic obstructive pulmonary disease (COPD), diabetic retinopathy and ischaemic or reperfusion injury [161]. Novel pyridine derivatives of type 58 and the use of the compounds in methods for treating conditions associated with at least one TK such as CDK, EMT, FAK, HER2, IGF-1R, IR, LCK, MET, PDGF or VEGF, including cancer and other proliferative diseases, are claimed. The ability of the compounds to bind to various receptors was measured *in vitro*. The compounds are stated to have IC_{50} values of < 25 μ M for the CDK, EMT, FAK, Her1, Her2, IGF, IR, LCK, MET, PDGF and VEGF receptors. They

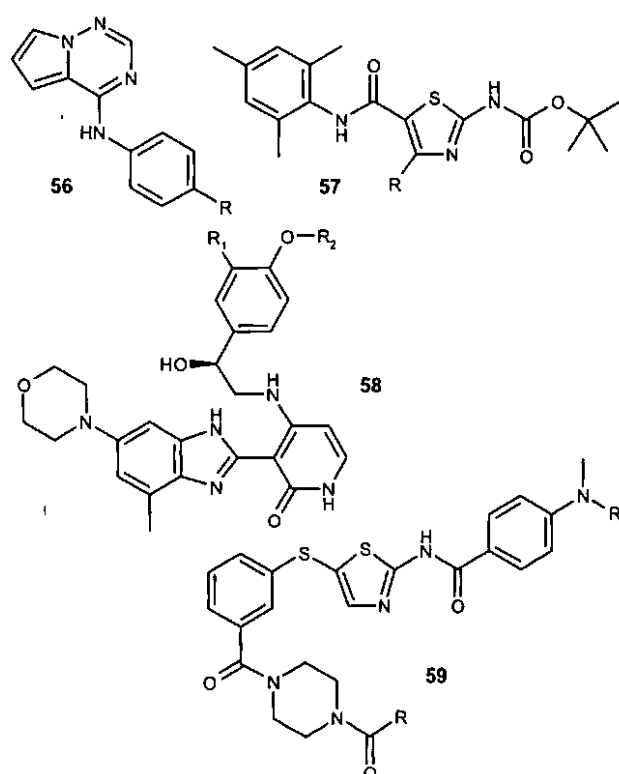


Figure 9. Protein tyrosine kinase inhibitors from Bristol-Myers Squibb.

also showed IC_{50} values ranging 0.1 – 1.4 μ M against HT-29, MCF-7 and Colo205 cells [162]. Novel 2-amino-5-(phenylthio)thiazole derivatives (59), their diastereomers and enantiomers, compositions containing them and optionally a further therapeutic agent and their use to treat Tec family TK-associated disorders were claimed. Methods of their use to treat Emt-associated disorders, transplant rejection, multiple sclerosis, inflammatory bowel disease, lupus, cancer, contact and atopic dermatitis, asthma, psoriasis, rheumatoid arthritis, graft-versus-host disease, T-cell-mediated hypersensitivity, Hashimoto's thyroiditis, Guillain-Barre syndrome, allergy, allergic rhinitis, ischaemic or reperfusion injury and COPD were also claimed [163].

2.8 Pfizer

Substituted bicyclic heteroaryl derivatives of type 60 (Figure 10) and methods of their use for treating various cancers, were claimed. These compounds are stated to be potent inhibitors of the erbB family of oncogenic and proto-oncogenic PTKs but no data are presented [164]. Novel heteroaromatic bicyclic derivatives of type 61 and processes for their preparation were claimed. The compounds were stated to be potent inhibitors of the erbB family of oncogenic and proto-oncogenic PTKs such as EGFR, erbB2, HER3 or HER4 and are further claimed for use in the prevention and treatment of a variety of disorders such as malignant and benign tumours of the liver, kidney, bladder, breast, ovary, colon, prostate, pancreas, lung, vulva and thyroid, hepatic carcinomas, sarcomas, glioblastomata, head and neck tumours and other hyperplastic conditions but no biological data are presented [165]. Heterocyclic compounds of type 62 and methods of their use in the treatment of angiogenesis disorders such as cancer and other hyperproliferative diseases, including prostate hyperplasia, optionally in combination with a further anticancer compound, are claimed. The compounds are stated to be inhibitors of RTKs. Assays for measuring the inhibition of KDF/VEGF receptors, KDR TK activity, mitogenesis in human endothelial cells and tumour growth are described but no data are presented [166]. Novel substituted bicyclic derivatives of type 63 and the use of the compounds in a method for treating abnormal cell growth including various forms of cancer, were claimed. These compounds are stated to be potent inhibitors of the erbB family of PTKs, in particular erbB2. The ability of the compounds to inhibit c-erbB2 kinase activity is described. The compounds are stated to have shown IC_{50} values of < 10 μ M but no specific data are presented. The ability of the compounds to inhibit tumour growth *in vivo* is also described but no resultant biological data are presented [167]. Novel 2,4-diaminopyrimidine derivatives, of type 64 and methods of their use for the inhibition of PTK Lck and for the treatment of a range of T-cell-mediated disorders, including transplant rejection, inflammatory disease, autoimmune disease, allergy, T cell leukaemia and T cell lymphoma, are claimed. The ability of the compounds to inhibit Lck-mediated phosphorylation was determined *in vitro*. IC_{50} values of < 3 μ M are stated to be preferred but no specific biological data were presented [168].

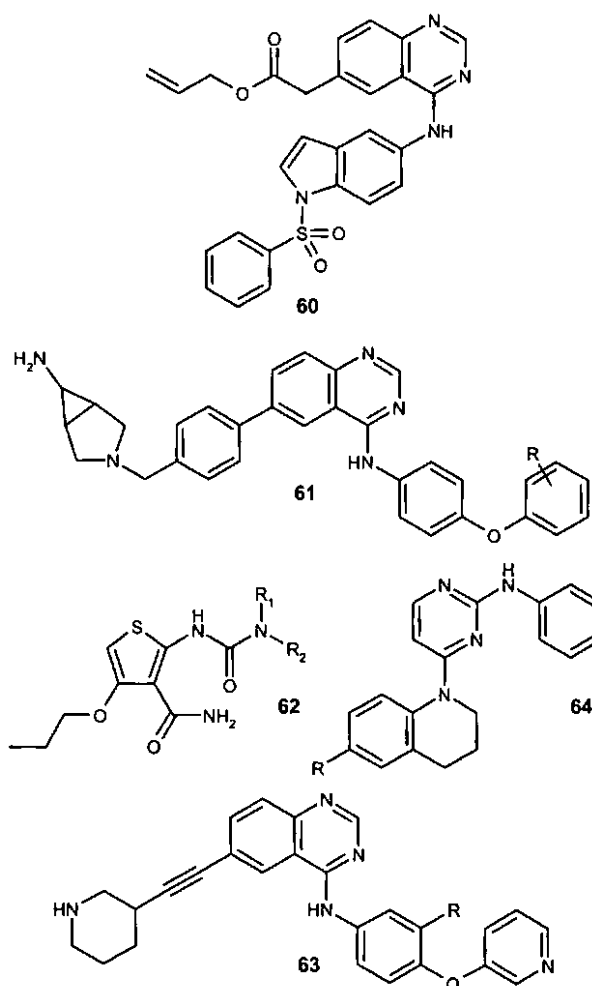


Figure 10. Protein tyrosine kinase inhibitors from Pfizer.

2.9 Boehringer Ingelheim

Novel substituted heterocycle derivatives of types 65 – 68 (Figure 11) were claimed by Boehringer Ingelheim. These compounds are stated to have inhibitory effects on a range of kinases, especially CDKs, viral cyclin and RTKs and they can therefore influence cell proliferation. They are further stated to be potentially useful for the treatment of a wide range of proliferative diseases, including viral infections (e.g., HIV and Kaposi's sarcoma), inflammation and autoimmune disease (e.g., colitis, arthritis, Alzheimer's disease, glomerulonephritis and wound healing), bacterial, fungal and parasitic infections, leukaemia, lymphoma and solid tumours. In addition, they may be useful for the protection of proliferating cells against DNA damage caused by radiation, ultraviolet (UV) treatment and/or chemotherapy. The ability of five compounds to inhibit the proliferation of cultivated SK-UT-1B leiomyosarcoma tumour cells was determined *in vitro*. These compounds exhibited IC_{50} values in the range 0.001 – 0.049 μ M [169-172]. The novel use of EGFR inhibitors of type 69 for the treatment

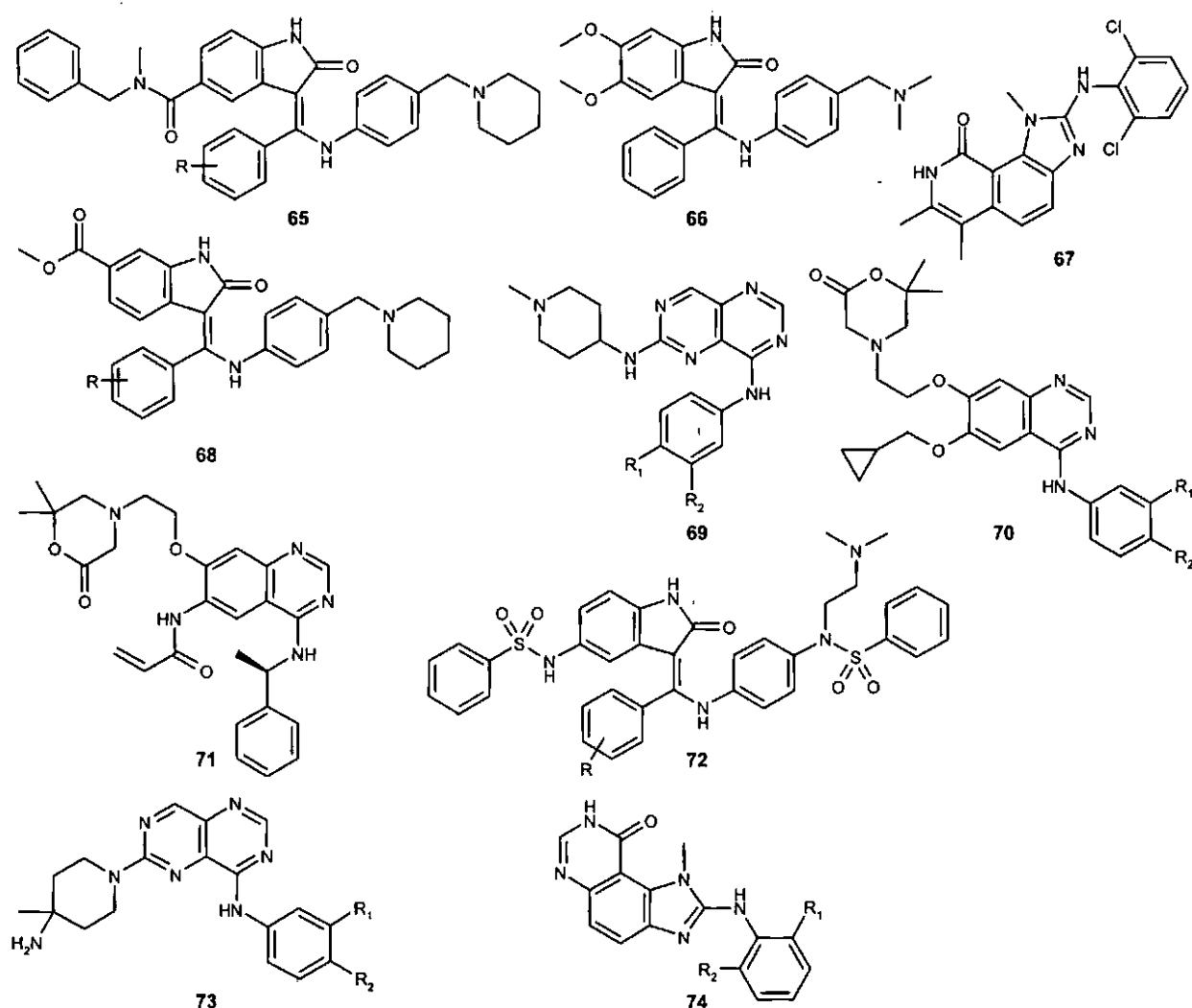


Figure 11. Protein tyrosine kinase inhibitors from Boehringer Ingelheim.

of tumours whose cells proliferate as a result of a deregulated mitogenic signal transduction pathway, particularly a deregulated Ras pathway, and require the function of wild type EGFR as a survival factor and as an inhibitor of differentiation, has also been claimed by the same group. The use of the EGFR inhibitors is claimed where the deregulation of the Ras pathway is the result of an oncogenic Ras mutation [173]. Novel quinazoline derivatives 70 and 71 and their use for the treatment of malidiseases, polyps, gastrointestinal diseases, gallbladder diseases, renal diseases and dermatological diseases, were then claimed. These compounds are stated to inhibit signal transduction mediated by the EGF receptor, by inhibiting the attachment of the growth factor to the receptor, receptor dimerisation, or later steps involved in signal transduction. The inhibitory activity of four compounds against EGFR-mediated signal transduction was evaluated *in vitro* using F/L-HERc cells expressing functional human EGFR. The IC_{50} values for the inhibition of EGF-dependent proliferation ranged from 29 to 59 nM [174,175]. Novel sulfonamido-substituted

3-(aminomethylidene)indolin-2-one derivatives of type 72, useful for inhibiting the proliferation of tumour and endothelial cells and for the treatment of diseases characterised by excessive cell proliferation, were also claimed. These compounds are disclosed to be inhibitors of various protein kinases, such as receptor and non-RTKs (including VEGF, EGF and IGF receptors or c-Src) or serine/threonine kinases (including cyclin-dependent kinases). An *in vitro* test for determining the ability of the compounds to inhibit proliferation of human leiomyosarcoma tumour cell lines, based on their ability to inhibit the integration of radiolabelled thymidine into cellular DNA, is described. A total of 34 compounds were tested, 13 of which showed IC_{50} values of < 0.01 μ M. An *in vivo* experiment for determining the effect of the compounds on mice carrying implanted or injected tumour cells is described but no resultant data are presented [176]. A novel process for the preparation of 4,6-diaminopyrimido[5,4-d]pyrimidine derivatives (73), comprising the removal of an N-protecting group, the reductive elimination of a chlorine substituent and the

oxidation of a suitable [6-substituted-2-chloro-7,8-dihydropyrimido[5,4-d]pyrimidin-4-yl]-substituted amine, was claimed. It is stated that 4,6-diamino-pyrimido[5,4-d]pyrimidine derivatives exhibit anticancer activity, are useful for the treatment of skin tumours and act by inhibiting TK [177]. Novel substituted heterocyclic derivatives of type 74 and a method for treating autoimmune diseases, neoplasm, inflammation, cerebral ischaemia and cardiovascular diseases are claimed. The compounds are stated to be useful for treating allergic diseases and transplant rejection and are also stated to be PTK inhibitors. *In vitro* TK inhibition was assessed against p56lck, p60 and PDGFR. All the compounds tested showed IC₅₀ values of < 10 µM against the three kinases. Inhibition of IL-2 production was also evaluated *in vitro*. Representative compounds showed IC₅₀ values of < 10 µM [178].

2.10 Various other protein tyrosine kinase inhibitors

Several other drug houses, universities and other research organisations have claimed many structural types of PTKIs and their use as antitumour agents. Most of them will be reviewed in this section. Xenova claimed novel benzofluoranthene derivatives of type 75 (Figure 12a), obtained by fermentation from the fungal strain *Cladosporium cladosporioides* X20700 (CBS 100220) and chemical processes for preparing them and their use in the treatment of cancer and immunoinflammatory conditions selected from rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, inflammatory bowel disease, Crohn's disease, systemic lupus erythematosus, multiple sclerosis, diabetes and asthma. These compounds were stated to inhibit TKs and the production of cytokines [179]. Novel 4-aminopyrrolopyrimidine derivatives of types 76 and 77 and methods of inhibiting protein kinase activity, particularly KDR, FGFR-1, PDGFRβ, PDGFRα, IGF-1R, c-Met, Fit-1, TIE-2, Lck, Src, fyn, Lyn and Blk and of treating a wide range of diseases including Kaposi's sarcoma, Hodgkin's disease, lymphoma, myeloma, leukaemia, Type 1 diabetes, glaucoma, diabetic retinopathy, ulcers, Crohn's disease, rheumatoid arthritis, HIV and multiple sclerosis, comprising the administration of these compounds, were claimed by BASF. The compounds are stated to inhibit the formation of oedema in an *in vivo* uterine oedema model in mice and to inhibit protein kinases such as cdc2 *in vitro* at < 50 µM but no data are presented. The compounds are further stated to inhibit the growth of primary murine, rat or human xenograft tumours in mice or inhibit metastasis in murine models [180,181]. Novel derivatives of types 78 and 79 and methods of treating neoplasms of the breast, kidney, bladder, mouth, larynx, oesophagus, stomach, colon, ovary and lung, as well as polycystic kidney disease, were claimed by American Cyanamid. The compounds are stated to inhibit the TK activity of KDR [182,183]. Schering claimed anthranilic acid amides of type 80 and their use for treating diseases sensitive to inhibition of angiogenesis. These compounds are disclosed to be angiogenesis inhibitors and to inhibit the TKs KDR and FLT, which form

part of the signalling pathway of VEGF receptors and are claimed for the treatment of many diseases mediated by angiogenesis, including tumours, psoriasis, arthritis, haemangioma, angiofibroma, a range of ocular, renal and fibrotic diseases, nerve injury, restenosis and atherosclerosis [184]. BASF claimed 2-pyrazolin-5-one derivatives of type 81 and their use for inhibiting protein kinase, particularly tyrosine and serine/threonine kinase activity, useful for treating a range of conditions including hyperproliferative disorders, inflammatory disease, angiogenesis, cancer, arthritis, restenosis, atherosclerosis, psoriasis, haemangioma, ARDS, stroke, burns and trauma and for affecting vascular hyperpermeability or the production of oedema [185]. Cephalon claimed pyrazolone derivatives of type 82 and methods of their use as trk, VEGFR, MLK and FGFR kinase inhibitors for treating or preventing angiogenic disorders, tumours, endometriosis, diabetic retinopathy, psoriasis, hemangioblastoma, ocular disorders, macular degeneration, Alzheimer's disease, amyotrophic lateral sclerosis, AIDS dementia, Parkinson's disease, stroke, ischaemia, Huntington's disease, epilepsy, multiple sclerosis, peripheral neuropathy, brain or spinal cord injury, restenosis, osteoporosis, inflammation, viral infection, bone or haematopoietic diseases, autoimmune diseases and transplant rejection. The VEGFR1 and 2, trkA, MLK-1, -2, -3 and FGFR1 kinase inhibitory activity of these compounds was investigated at 1 µM. The obtained results show that the specified compound showed the best overall results, inhibiting VEGFR1 and 2, trkA, MLK-1, -2, -3 and FGFR1 by 74, 88, 53, 44, 17, 72 and 48%, respectively [186]. Takeda claimed a novel process for the production of 1-substituted-1,2,3-triazoles (83) for obtaining TK inhibitors but no biological data are provided [187]. Processes for the regioselective preparation of derivatives (84) from 3-aminonaphthalene-2-carboxylates and 3-aminonaphthalene-2-carbonitriles, as TK inhibitors useful as anticancer agents were claimed by Wyeth [188]. TK inhibitors comprising shikonin (compound 85) or its derivatives having a naphthoquinone skeleton as the active component were claimed by Showa Univ. Medicinal compositions comprising these compounds for the treatment of cancers, including breast, colon, lung, ovary and stomach, were also claimed. The shikonin derivatives include β-dimethylacrylshikonin, isovalerylshikonin, α-methyl-N-butylshikonin, isobutylshikonin, shikonin and β-hydroxyisovalerylshikonin were claimed to act as PTKIs [189]. Abbott claimed derivatives of type 86 and their use as protein kinase inhibitors, particularly serine/threonine or PTK inhibitors. The compounds are claimed for use for the treatment of hyperproliferative disorders, ulcers, ocular disease, cardiovascular disease, neoplasm, Crow-Fukase syndrome, diabetic conditions, sickle cell anaemia, inflammation, systemic lupus erythematosus, glomerulonephritis, synovitis, inflammatory bowel disease, Crohn's disease, multiple sclerosis, rheumatoid arthritis, osteoarthritis, graft rejection, Lyme disease, sepsis, Von Hippel Lindau disease, pemphigoid, psoriasis, Paget's disease, polycystic renal disease, fibrosis, sarcoidosis,

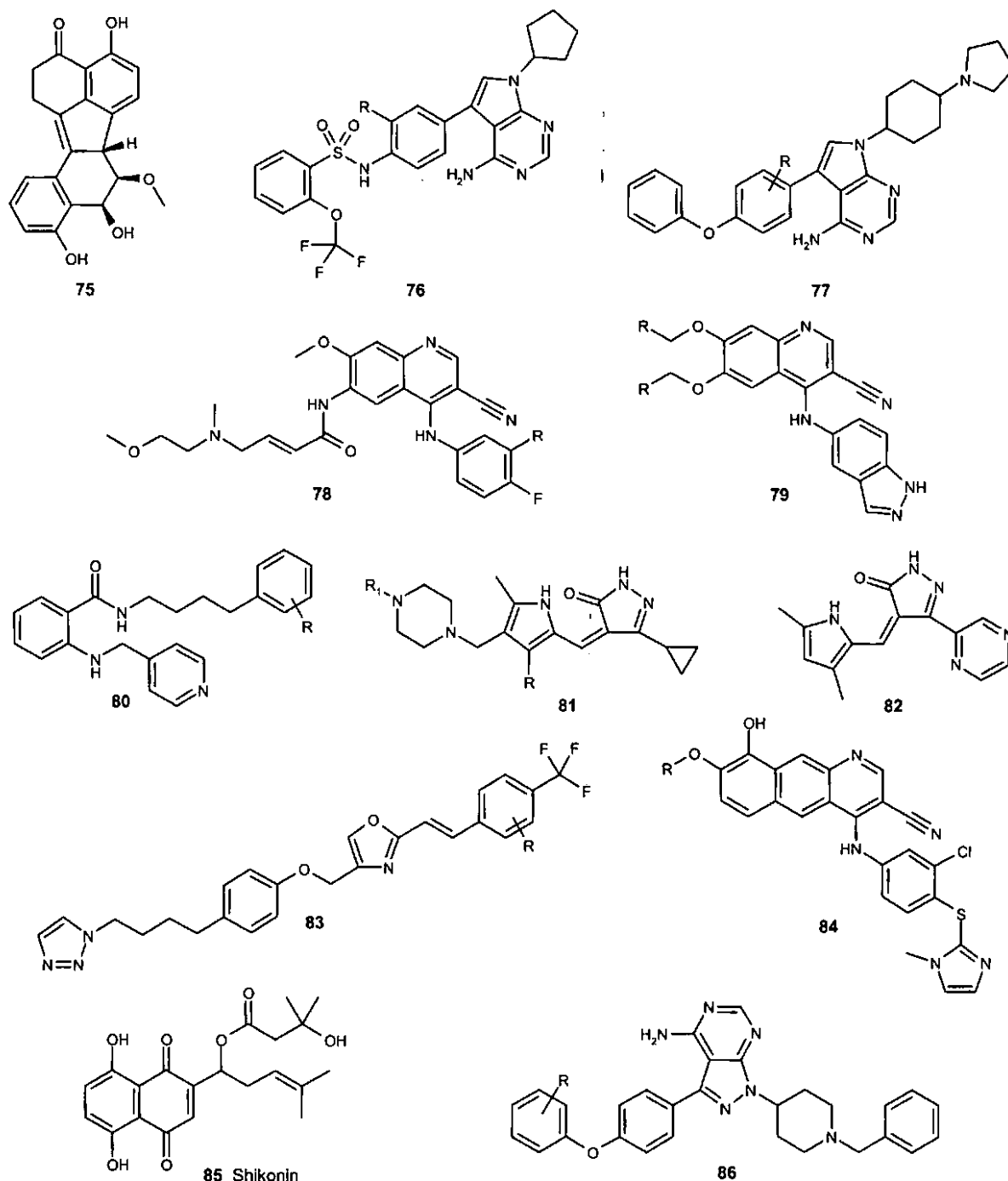


Figure 12a. Various other protein tyrosine kinase inhibitors.

cirrhosis, thyroiditis, COPD, asthma, oedema, injury, radiation sickness, cerebrovascular ischaemia, hypoxia, ovarian hyperstimulation, pre-eclampsia, endometriosis, menometrorrhagia, herpes virus infection, HIV infection and *Toxoplasma* infection and for decreasing fertility. The use of the compounds, optionally in combination with a pro-angiogenic

growth factor, to promote angiogenesis is also claimed. The compounds were tested for the inhibition of phosphorylation of an exogenous substrate by a range of protein kinases. It was disclosed that the compounds inhibit FGFR, PDGFR, KDR, Tie-2, Src, Lck, Fyn, Btk and CDC2 at concentrations of < 50 μ M but no specific biological data were

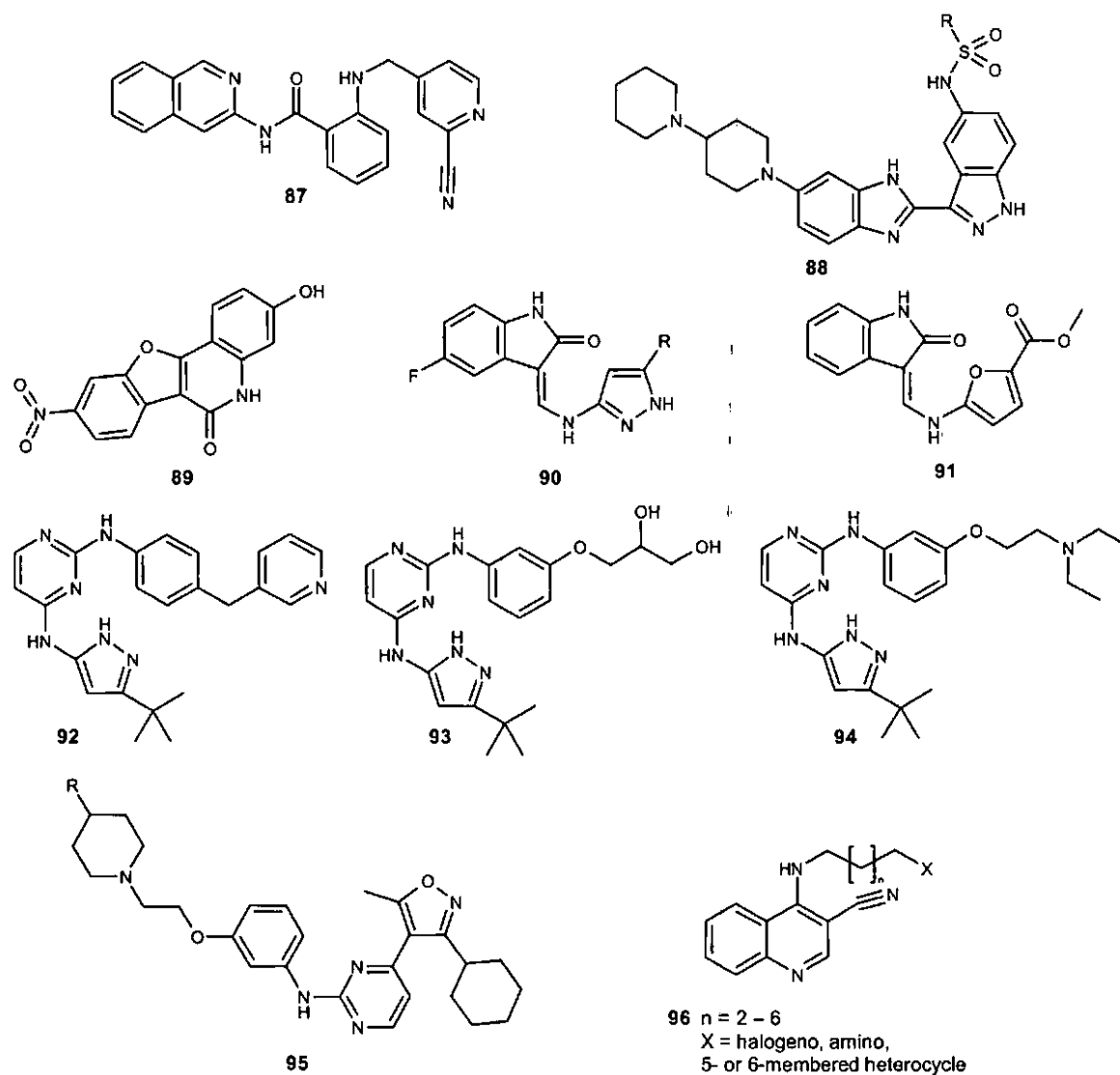


Figure 12b. Various other protein tyrosine kinase inhibitors.

presented. Some compounds also inhibited VEGFR-induced KDR phosphorylation in a cellular assay at $< 50 \mu\text{M}$ [190]. Novel 2-(cyanopyridylmethylamino)benzamide derivatives (87, Figure 12b) and their use as VEGFR3 inhibitors in lymphangiogenesis, KDR and FLT TK inhibitors and for the treatment of psoriasis, Kaposi's sarcoma, restenosis, endometriosis, Crohn's disease, Hodgkin's disease, leukaemia, arthritis, haemangioma, angiofibroma, age-related disorders, diabetic nephropathy, diabetic retinopathy, malignant nephrosclerosis, thrombotic and fibrotic disorders, kidney and eye diseases and for inhibition of reocclusion after balloon catheter treatment and in vascular prosthetics or implants were claimed by Schering. The ability of the compounds to inhibit KDR- and FLT-1 kinase activity is provided in one example. The compounds showed IC_{50} values of $0.01 - 0.7 \mu\text{M}$ for the

inhibition of VEGFR II (KDR) [191]. Novel 8-(indazol-3-yl)purine and 2-(indazol-3-yl)benzimidazole derivatives of type 88 were claimed by Chiron. These compounds are stated to be useful as inhibitors of tyrosine and serine/threonine kinases and for the treatment of cancer, non-insulin-dependent diabetes and CNS disorders, including Alzheimer's disease and as inhibitors of angiogenesis. Assay protocols for determining inhibition of the flt-1, Flk-1, Tie-2, PDGF and bFGF TKs and serine/threonine kinases, such as GSK-3 and CHK-2 were described. Certain of the exemplified compounds are stated to display IC_{50} values of $< 1 \mu\text{M}$ but no specific data are presented [192]. Novel heterocyclic protein kinase inhibitors, among which derivative 89 and methods of their use for the inhibition of a cell proliferative or skeletal disease caused by abnormal protein kinase activity, for

the inhibition of tumour angiogenesis and for the treatment of non-solid cancers or solid tumours, bone and cartilage-related disorders, skeletal dysplasia or craniosynostosis disorders were claimed by ProChon Biotech. The efficacy of the compounds was assessed in a cell proliferation assay for the inhibition of FDFR3. Compound 89 showed an IC_{50} value of 7 μ M. The effect of one of the compounds (not the specified compound) on the growth rate of G396C achondroplasia heterozygote mice femora bone in organic culture was also evaluated [193]. Allergan claimed novel 3-(heteroaryl-amino)methylene-1,3-dihydro-2H-indol-2-one derivatives 90 and 91 (similar to the Sugen lead compound 5 discussed earlier) and their use in methods for the treatment of unregulated TK signal transduction, including cancer, blood vessel proliferative disorders (e.g., diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, arthritis and restenosis), fibrotic disorders (e.g., hepatic cirrhosis and atherosclerosis), mesangial cell proliferative disorders (e.g., glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection and glomerulopathies) and metabolic diseases (e.g., psoriasis, diabetes mellitus, wound healing, inflammation and neurodegenerative diseases) are claimed. Several assays are described. In one assay (the Miles assay), VEGF-induced dermal extravasation was determined in guinea-pigs. Two compounds, tested at a dose of 75 mg/kg, produced 83 or 100% inhibition [194,195]. In a group of patents, Bayer claimed new 2-phenylamino-4-(pyrazol-5-ylamino)pyrimidine derivatives of types 92 – 94 and their use in methods for inhibiting Src kinase receptors, in particular for treating osteoporosis, melanoma, Kaposi's sarcoma and breast, colon, pancreatic, lung, neural, oesophageal and gastric cancers and for treating non-malignant proliferative disease. The activity of the compounds was measured in a biochemical Src kinase assay *in vitro*. Approximately 50 compounds, were stated to have shown IC_{50} values of < 500 nM in this assay. No specific data are presented [196-198]. Novel isoxazolympyrimidine compounds of type 95 and methods of their use as inhibitors of Src or Lck protein kinases and for the treatment of hypercalcemia, restenosis, osteoporosis, osteoarthritis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, psoriasis, lupus erythematosus, graft-versus-host disease, hypersensitivity, Hashimoto's thyroiditis, Guillain-Barre syndrome, COPD,

dermatitis, atopic dermatitis, cancer, bone metastasis, Paget's disease, asthma, ischaemic or reperfusion injury, allergy, allergic rhinitis, autoimmune diseases and leukaemia were claimed by Vertex. The compounds were assayed for inhibition of human Src and Lck kinases using either radioactivity-based or spectrophotometric assays. Ten compounds, including the specified compound, had inhibition constant (K_i) values of < 0.1 μ M for inhibition of Src kinase and 32 compounds, including the specified compound, had K_i values of < 0.1 μ M for inhibition of Lck kinase. No specific data are presented [199]. Wyeth also claimed EGFR and HER-2 PTKIs, belonging to the class of 3-cyano-quinolines and 3-cyano-1,7-naphthyridines 96 (related to compound 84 mentioned previously) and their use as antineoplastic agents or for the treatment of polycystic kidney disease [200-204].

3. Expert opinion

As receptor and non-receptor PTKs are essential enzymes in cellular signalling processes, regulation of cell growth, differentiation, migration and metabolism, and because the kinase activity of PTKs is tightly controlled through steric, autoregulatory mechanisms, as well as by the action of protein tyrosine phosphatases, its inhibition has only recently been proven to lead to effective anticancer therapies [1-9]. Indeed, the aberrant catalytic activity of many PTKs, via mutation or overexpression, plays an important role in numerous pathological conditions, the most important of which is cancer [1-9]. The first clinically used drug belonging to the class of the PTKIs, imatinib (compound 1), has only recently (2001) been approved, but its clinical success is significant [2,6,7]. Thus, much research is being undertaken in this field in order to find both second generation compounds with improved activity, reduced toxicity, and improved selectivity, as well as derivatives targeting other kinases among the 700 – 800 encoded by the human genome. Indeed, as presented in this review, the major drug houses worldwide all have impressive drug development programmes in place for the discovery of novel classes of PTKIs. Furthermore, many patents deal with the use of such compounds in combination therapies for the treatment of a multitude of tumours. Thus, it may be stated that we are at the beginning of a new era in the design, synthesis and use of PTKIs for the treatment of cancer.

19

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