Pharmacological Research 129 (2018) 65–83

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Invited Review

The role of small molecule platelet-derived growth factor receptor (PDGFR) inhibitors in the treatment of neoplastic disorders

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ARTICLE INFO

Article history:
Received 28 January 2018
Accepted 29 January 2018
Available online 3 February 2018

Chemical compounds studied in this article:
Axitinib: (PubMED CID: 6450551)
Dasatinib: (PubMED CID: 3062316)
Imatinib: (PubMED CID: 5291)
Lenvatinib: (PubMED CID: 9823820)
Nilotinib: (PubMed CID: 644241)
Nintedanib: (PubMed CID: 9809715)
Ponatinib: (PubMed CID: 24826799)
Sorafenib: (PubMed CID: 216239)
Sunitinib: (PubMed CID: 5329102)

Keywords:
Catalytic spine
K/E/D/D
Protein kinase inhibitor classification
Protein kinase structure
Regulatory spine
Targeted cancer therapy

ABSTRACT

Platelet-derived growth factor (PDGF) was discovered as a serum-derived component necessary for the growth of smooth muscle cells, fibroblasts, and glial cells. The PDGF family is a product of four gene products and consists of five dimeric isoforms: PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and the PDGF-AB heterodimer. This growth factor family plays an essential role in embryonic development and in wound healing in the adult. These growth factors mediate their effects by binding to and activating their receptor protein-tyrosine kinases, which are encoded by two genes: *PDGFRA* and *PDGFRB*. The functional receptors consist of the PDGFRα/α and PDGFRβ/β homodimers and the PDGFRα/β heterodimer. Although PDGF signaling is most closely associated with mesenchymal cells, PDGFs and PDGF receptors are widely expressed in the mammalian central nervous system. The PDGF receptors contain an extracellular domain that is made up of five immunoglobulin-like domains (Ig-D1/2/3/4/5), a transmembrane segment, a juxtamembrane segment, a protein-tyrosine kinase domain that contains an insert of about 100 amino acid residues, and a carboxyterminal tail. Although uncommon, activating mutations in the genes for PDGF or PDGF receptors have been documented in various neoplasms including dermatofibrosarcoma protuberans (DFSP) and gastrointestinal stromal tumors (GIST). In most neoplastic diseases, PDGF expression and action appear to involve the tumor stroma. Moreover, this family is pro-angiogenic. More than ten PDGFRα/β multikinase antagonists have been approved by the FDA for the treatment of several neoplastic disorders and interstitial pulmonary fibrosis (www.brimr.org/PKI/PKIs.htm). Type I protein kinase inhibitors interact with the active enzyme form with DFG-D of the proximal activation segment directed inward toward the active site (DFG-D_{in}). In contrast, type II inhibitors bind to their target with the DFG-D pointing away from the active site (DFG-D_{out}). We used the Schrödinger induced-fit docking protocol to model the interaction of several antagonists with PDGFRα including imatinib, sorafenib, and sunitinib. The results indicate that these antagonists are able to bind to the DFG-D_{out} conformation of the receptor and are thus classified as type II inhibitors. Owing to the multiplicity of less active protein kinase conformations when compared with the canonical more active conformation, it was hypothesized that type II drugs would be less promiscuous than type I drugs which bind to the typical active conformation. Although type II inhibitors may be more selective, most – if not all – inhibit more than one target protein kinase and the differences are a matter of degree only.

Contents

Abbreviations: AS, activation segment; CS or C-spine, catalytic spine; CL, catalytic loop; CTT, carboxyterminal tail; DFSP, dermatofibrosarcoma protuberans; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GIST, gastrointestinal stromal tumors; GK, gatekeeper; GRL, Gly-rich loop; Ig-D, immunoglobulin-like domain; KD, kinase domain; KID, kinase insert domain; JM, juxtamembrane; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; PH, pleckstrin homology; PI–3 K, phosphatidylinositol 3′-phosphate kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTB, phosphotyrosine binding; pY or pTyr, phosphotyrosine; RS or R-spine, regulatory spine; Sh2, shell residue 2; SH2, Src homology 2; TM, transmembrane; VEGFR, vascular endothelial growth factor receptor.

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https://doi.org/10.1016/j.phrs.2018.01.021
1043-6618/

1. Overview of the platelet-derived growth factor ligands and receptors

1.1. Structure and biosynthesis of the PDGF family ligands

Platelet-derived growth factor (PDGF) was discovered in the 1970s as a serum-derived component necessary for the growth of smooth muscle cells, fibroblasts, and glial cells [1–3]. The PDGF family is a product of four gene products (Table 1) and consists of five dimeric isoforms: PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and the PDGF-AB heterodimer [4]. The B-chain was characterized by amino acid sequencing [5,6] and the A-chain was characterized by cDNA sequence analysis [7]. The PDGF-CC and DD factors were discovered nearly two decades later [8,9]. This growth factor family plays an essential role in embryonic development and in wound healing in the adult [4]. It is likely that these factors have additional roles in adults, but the nature of these functions is unclear. This growth factor family mediates their effects in target cells by binding to and activating their receptor protein-tyrosine kinases: PDGFRα and PDGFRβ. The functional receptors consist of the PDGFRα/α and PDGFRβ/β homodimers and the PDGFRα/β heterodimer. The PDGF receptors are most closely related to the Flt3 and the stem cell factor receptor (Kit) protein-tyrosine kinases [10].

PDGF-AA, PDGF-BB, and PDGF-AB are synthesized by many different cell types [11]. These include, but are not limited to, fibroblasts, Leydig cells, kidney mesangial cells, skeletal myoblasts, vascular smooth muscle cells, vascular endothelial cells, astrocytes, neurons, Schwann cells, retinal pigment epithelial cells, macrophages, platelets, and megakaryocytes. These same cells express PDGFRα, PDGFRβ, or both. Thus, PDGF signaling may be autocrine in nature. Keratinocytes, oöcytes, uterine endometrial and myometrial cells, and mammary epithelial cells produce PDGF-AA, PDGF-BB, and PDGF-AB, but not PDGF receptors. In contrast, T cells and myeloid hematopoietic cells produce PDGFRβ, but not PDGF-A or B-chains. Most cell types expressing these growth factors produce both A- and B-chains; however, their expression is independently regulated at both the transcriptional and post-transcriptional levels.

Although PDGF signaling is most closely associated with mesenchymal cells, PDGFs and PDGF receptors are widely expressed in the mammalian central nervous system [12,13]. Sasahara et al. demonstrated that the PDGF-B chain was expressed throughout the brain, in the dorsal horn of the spinal cord, and in the posterior pituitary of a nonhuman primate (*Macaca nemestrina*) [12]. It was expressed in neuronal cell bodies – but not in the axons or nerve terminals – of the cortex, hippocampus, amygdala, thalamus, caudate, globus pallidus, putamen, and substantia nigra. The PDGF-B chain was expressed in the cell bodies, axons, and terminals of the posterior pituitary, the supraoptic and paraventricular hypothalamus, and the dorsal horn of the spinal cord. The PDGF-A chain is expressed in glial cells, but these cells fail to express PDGF-B chains [12]. Ishii et al. found that the expression of PDGFRβ in mouse neurons protected these cells from cryogenic injury and *N*-methyl-D-aspartate (NMDA) excitotoxicity [13]. Owing to the potential importance of PDGF signaling in brain, the effect of PDGFR antagonists on neuronal function and toxicity needs to be addressed.

The PDGF isoforms are synthesized as preproproteins that are subsequently proteolytically processed into mature forms [4]. The signal sequences of PDGF-AA, PDGF-BB, and PDGF-AB are cleaved in reactions mediated by signal peptidases. The dimeric forms of the growth factors are linked by two disulfide bonds. Proprotein convertases catalyze the removal of an amino-terminal pro sequence to yield the mature isoform, which is then secreted. In contrast, PDGF-CC and PDGF-DD are secreted as inactive forms following the removal of their signal peptides. PDGF-CC is processed extracellularly by the tissue-type of plasminogen activator (tPA) or by plasmin to yield its active form and PDGF-DD is processed extracellularly by the urokinase-type of plasminogen activator (uPA) or by matripase to produce its active form [14]. These proteases catalyze the hydrolytic removal of an amino-terminal CUB domain (Complement proteins C1r/C1s, Uegf, Bmp1) yielding the active form of these two growth factors.

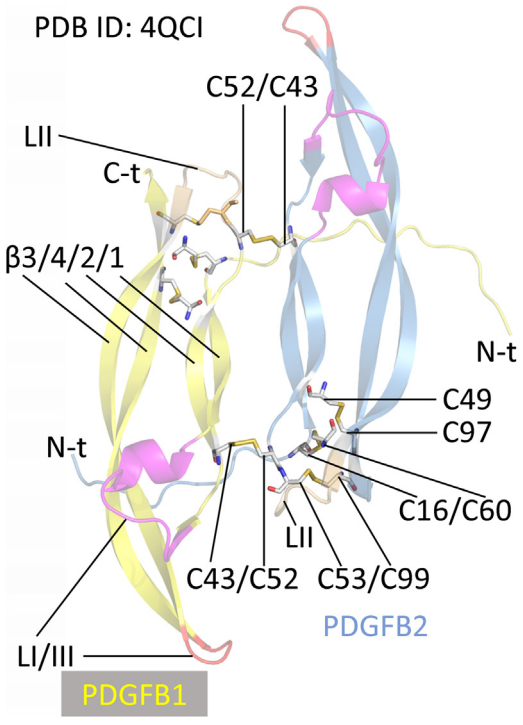

Fig. 1. Secondary structure of PDGF-BB. The C43 and C52 disulfide bridges connect the two protomers. C-t, C-terminus; L, loop; N-t, N-terminus. All figures except for 2, 7, and 9 were prepared using the PyMOL Molecular Graphics System Version 1.5.0.4 Schrödinger, LLC.

Table 1
Properties of the platelet-derived growth factor components.

Factor	UniProt ID	No. of residues	Signal sequence residues	Residues in the mature factor	Selected functions
PDGFA	P04085	211	1–20	87–211	Potent mitogen for cells of mesenchymal origin. Plays an important role in wound healing. Required for lung, gastrointestinal, and oligodendrocyte development and normal myelination of the spinal cord and cerebellum.
PDGFB	P01127	241	1–20	82–190	Potent mitogen for cells of mesenchymal origin. Participates in normal blood vessel and kidney glomerular development. Necessary for normal proliferation of pericytes and vascular smooth muscle cells in the central nervous system, heart, lung, placenta, and skin.
PDGFC	Q9NRA1	345	1–22	238–345	Required for formation of the skeleton especially the craniofacial bones and palate. Necessary for normal skin morphogenesis, angiogenesis, and blood vessel development.
PDGFD	Q9GZP0	370	1–18	250–370	Induces macrophage recruitment, blood vessel maturation, and plays a role in the regulation of interstitial pressure.

Oefner et al. determined the X-ray crystal structure of the dimeric human PDGF-BB [15]. Each polypeptide chain has two long twisted antiparallel pairs of β-strands. These strands are connected by loops I (I25–L38), II (C53–V58), and III (V78–K81) (Fig. 1). Eight cysteine residues are conserved in each protomer. The second and fourth cysteines form a disulfide bridge between the two subunits of the dimer: C43 of one protomer bonds with C52 of the other protomer and vice versa. Each protomer contains an intramolecular cystine cluster that is located in the region of loop II. The first cysteine pairs with the sixth cysteine, the third pairs with the seventh, and the fifth pairs with the eighth. Thus, the three disulfide bonds encompass C16–C60, C49–C97, and C53–C99. These linkages make up a knot with C16–C60 penetrating the cyclic structure formed by the other two disulfide bonds. The secondary structure of PDGF-BB is similar to that of VEGF-A, which has a related amino acid sequence, and it resembles that of nerve growth factor and transforming growth factor-β, both of which lack sequence homology with the PDGFs.

PDGFRα/α, PDGFRα/β, and PDGFRβ/β are typical receptors that contain an extracellular domain that is made up of five immunoglobulin-like domains (Ig-D1/2/3/4/5), a transmembrane segment, a juxtamembrane segment, a protein-tyrosine kinase domain that contains an insert of about 100 amino acid residues, and a carboxyterminal tail [16–18]. These receptors were initially characterized by cDNA sequence analysis in the 1980s. As discussed in detail later, the growth factors promote the dimerization of two receptor monomers that leads to the stimulation of their intrinsic protein kinase activity and results in the phosphorylation of several receptor tyrosines that play key roles in signal transduction. PDGF-AA, PDGF-AB, PDGF-BB, and PDGF-CC promote PDGFRα/α dimer formation and PDGF-BB and PDGF-DD promote PDGFRβ/β dimer formation. In contrast, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-AB promote PDGFRα/β heterodimer formation (Fig. 2).

Shim et al. determined the X-ray crystal structure of PDGF-BB together with the first three immunoglobulin-like domains of PDGFRβ [19]. The PDGF-BB dimer links two PDGFRβ monomers together. Growth factor binding is limited to the Ig-D2 and Ig-D3 domains (Fig. 3). The core scaffold of PDGF-BB bound to the receptor closely resembles that of the free ligand. The loops of the growth factor interact with each receptor monomer: LI and LIII of the one protomer and LII of the second protomer interact with one receptor while LI and LIII of the second protomer and LII of the first protomer interact with a second receptor. The X-ray studies were in agreement with the results of site-directed mutagenesis that demonstrated that the most important residues involved in receptor binding occur in these loops [11]. The interactions between the ligand and receptor involve both hydrophobic interactions and electrostatic salt bridges [19].

1.2. PDGF receptor signaling

Cell signaling and signal transduction depend upon a variety of modular protein domains that participate in protein–protein or protein-lipid interactions [20,21]. The Src homology 2 (SH2) domain was the first to be characterized as a noncatalytic, or regulatory, component of the Src nonreceptor protein-tyrosine kinase. SH2 domains consist of about 100 amino acid residues and they bind to phosphotyrosine in a sequence-specific fashion depending upon the identity of amino acids C-terminal to the phosphotyrosine [22]. SH3 domains consist of about 60 amino acid residues and they bind preferentially to sequences that can adopt a left-handed helical conformation; such conformations are generally enriched in prolines (PxxP). Pleckstrin homology (PH) domains consist of about 110 amino acid residues that bind to membrane-associated phosphoinositides; this interaction attracts proteins to the plasma membrane. Phosphotyrosine binding (PTB) domains consist of about 110 amino acid residues that bind preferentially to a ΦxNPxpY motif where Φ is a hydrophobic residue and pY is phosphotyrosine. Moreover, it is not uncommon for a single protein to contain multiple modular domains. For example, phospholipase Cγ (PLCγ) contains two SH2, one SH3, and two PH domains [11].

Following the phosphorylation of numerous tyrosine residues within the PDGF receptor intracellular domain after dimerization, both enzymatically active and adaptor proteins bind to the receptor phosphotyrosines via their SH2 domains [20,21]; such binding to the PDGF receptors leads to alterations in gene expression and stimulation of downstream signaling modules that promote cell proliferation, migration, and survival (Fig. 2; Table 2) [14]. The regulatory subunit of phosphatidylinositol 3-kinase (PI–3 K), which contains two SH2 and one SH3 domain, binds to two phosphotyrosines in PDGFRα (pY731 and pY742) and two residues in PDGFRβ (pY740 and pY751) [11]. The PI–3K catalytic subunit is attracted to the regulatory subunit and is thereby activated. This enzyme family catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate to form phosphatidylinositol 3,4,5-trisphosphate. The trisphosphate binds to the PH domain of the Akt/PKB protein-serine/threonine kinase leading to its activation and thereby promoting cell survival by inhibiting apoptosis [23]. PLCγ binds to both PDGFRα (pY988 and pY1018) and PDGFRβ (pY1009 and pY1021) thereby leading to its activation [11]. PLCγ acts on the same substrate as PI–3 K leading to the hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5 trisphosphate and diacylglycerol [24]. The trisphosphate interacts with its receptor located in the endoplasmic reticulum membrane and liberates Ca^{2+} from intracellular stores allowing Ca^{2+} and diacylglycerol to act in concert to activate PKC. The PKC family, which consists of nine members, participates in many cellular processes including the regulation of cell growth, motility, and survival [25].

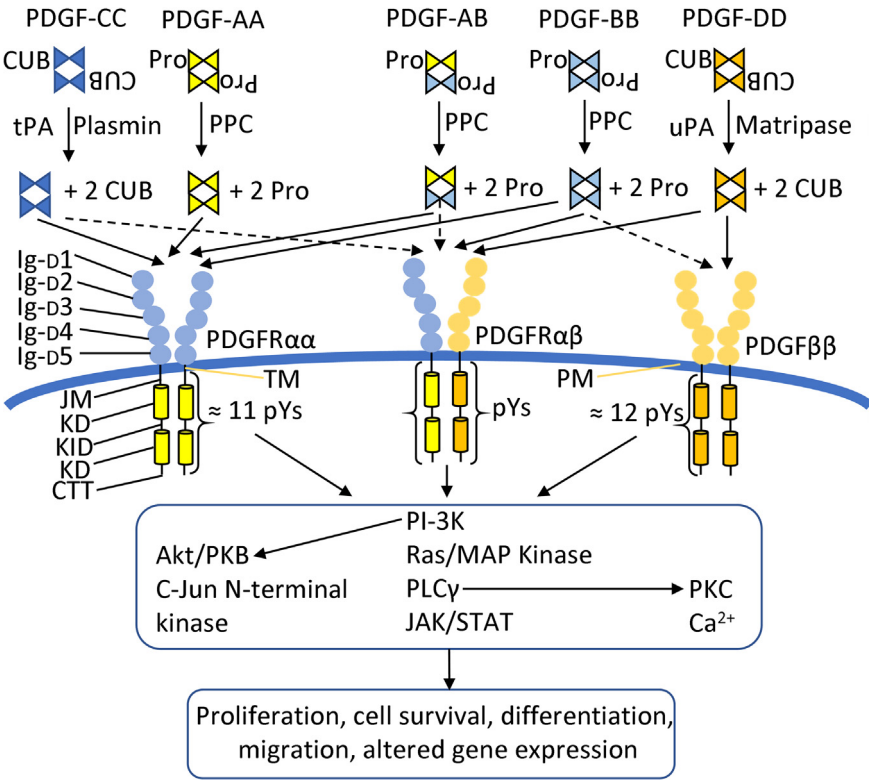

Fig. 2. Proteolytic processing of the platelet-derived growth factors, their interaction with PDGFRαα, PDGFRαβ, and PDGFRββ, and intracellular downstream signaling pathways. CTT, carboxyterminal tail; Ig-D, immunoglobulin-like domain; KD, kinase domain; KID, kinase-insert domain; JM, juxtamembrane; PM, plasma membrane; PPC, proprotein convertase, TM, transmembrane; tPA, tissue-type of plasminogen activator; uPA, urokinase-type of plasminogen activator.

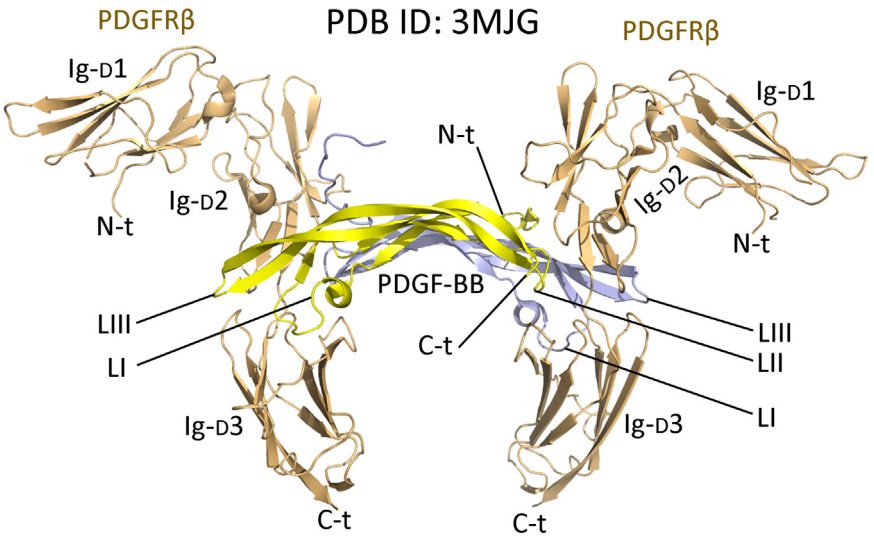

Fig. 3. Interaction of PDGF-BB (yellow and light blue) with the extracellular domain of PDGFRββ (tan). C-t, C-terminus; Ig-D immunoglobulin-like domain; L, loop; N-t, N-terminus.

The nonreceptor protein-tyrosine kinase Src with its SH2 domain binds to pY572 of PDGFRα and to pY579 of PDGFRβ [11]; Src served as the prototype for the study of SH2 and SH3 domains while the SH1 domain corresponds to its protein kinase component [20]. Src participates in many cellular processes including angiogenesis, cell proliferation, survival, and motility [11]. The Grb2 adaptor protein, which contains one SH2 and two SH3 domains, binds to pY716 and pY775 of PDGFRβ via its SH2 domain. Grb2 binds to Sos via its SH3 domains; Sos is a nucleotide exchange factor for Ras which leads to the conversion of inactive Ras-GDP to active Ras-GTP [26]. This response leads to the stimulation of the Ras/Raf/MEK/ERK cell proliferation pathway [27–29]. The protein-tyrosine phosphatase Shp-2 with two SH2 domains binds to two phosphotyrosines in PDGFRα (pY720 and pY754) and two residues in PDGFRβ (pY763 and pY1009). Following activation, the enzyme may catalyze the dephosphorylation of inhibitory Src phosphotyrosines leading to Src activation. Shp-2 may also bind and activate Grb2/Sos and thereby contribute to Ras activation [30]. The GTPase activating protein (Ras-GAP) binds to pY771 in PDGFRβ leading to the conversion of Ras-GTP to Ras-GDP and modulation of Ras activity [11]. The STAT5 factor, which contains one SH2 and one SH3 domain, binds to pY579, pY581, and pY775 of PDGFRβ. After its

Table 2
Tyrosine phosphorylation sites in PDGFRα/β[a].

Location[b]	PDGFRα pY	Interactions	PDGFRβ pY	Interactions
JM	572	Src	579	Shc, Src, STAT5
JM	574		581	Src, STAT5
KID	720	Shp2	716	Grb2
KID	731	PI–3K	740	PI–3K, Shc
KID	742	PI–3K	751	PI–3K, Shc, Nck
KID	754	Shp2	763	Shp2
KID	762	Crk	771	Ras-GAP, Shc
KID	768		775	Grb2, STAT5
KD-AS	849		857	
KD-αH-helix			934	
CTT	988	PLCγ	1009	PLCγ, Shp2
CTT	1018	PLCγ	1021	PLCγ

[a] Data from UniProt ID P16234, P09619, and Ref. [14].
[b] AS, activation segment; CTT, carboxyterminal tail; KD, kinase domain; KID, kinase insert domain; JM, juxtamembrane segment; pY, phosphotyrosine.

phosphorylation by the receptor, it dimerizes and is translocated to the nucleus where it functions as a transcription factor.

The adaptor protein Nck, which contains one SH2 domain and three SH3 domains, interacts with pY751 of PDGFRβ [11]. Nck participates in the activation of the C-Jun N-terminal protein-serine/threonine kinase (JNK) family encoded by three genes. The JNK family participates in the regulation of cell growth, differentiation, and survival. The adaptor protein Shc, which contains one SH2 and one PTB domain, binds to pY579, pY740, pY751, and pY771 of PDGFRβ and is phosphorylated at residue Y317; phosphorylated Shc binds to Grb2/Sos leading to stimulation of the Ras/MAP kinase pathway. The adaptor protein Crk, which contains one SH2 and two SH3 domains, binds to pY762 of PDGFRα and forms a complex with the Cas docking protein and the nucleotide exchange factor C3G; this interaction leads to the stimulation of the Janus kinase pathway [31]. As can be seen, PDGFRα/β participate in a myriad of signaling pathways. These pathways are also regulated by other receptors and there is extensive crosstalk among the various signaling modules. This crosstalk involves both stimulatory and inhibitory interactions and is exquisitely fine-tuned.

2. Dysregulation of the PDGF/PDGFR pathways in assorted diseases

Activating mutations in the genes for PDGF or PDGF receptors have been documented in a variety of neoplasms including dermatofibrosarcoma protuberans (DFSP), gastrointestinal stromal tumors (GIST), leukemias, and gliomas [4]. For example, DFSP results from the translocation of the *PDGFB* gene to the collagen *COL1A1* gene which leads to the production of a fusion protein containing the amino-terminal collagen sequences and the carboxyterminal PDGF-B sequence [32]. This fusion protein is proteolytically processed and a PDGF-like protein is released which stimulates skin fibroblasts and drives development of this rare disorder by an autocrine mechanism. About 95% of these tumors are low grade sarcomas and only 5% eventually metastasize. The 10-year survival rate is greater than 98% [33]. These neoplasms are treated surgically and the FDA has approved imatinib for the treatment of patients with unresectable or metastatic disease (www.brimr.org/PKI/PKIs.htm).

Cases of GIST represent the most common mesenchymal tumor, or sarcoma, of the gastrointestinal tract [34]. About 80–85% of these tumors result from activating mutations in the *KIT* proto-oncogene [35]. However, point mutations in the *PDGFRA* gene result in the production of an activated PDGFRα that occurs in about 5–7% of these neoplasms, which corresponds to 150–300 new cases in the United States per year [4,32,36]. The *KIT* and *PDGFRA* mutations are mutually exclusive. Heinrich et al. reported that the activating *PDGFRA* mutations occurred within (i) the JM segment (exon 12 V561D), (ii) the small lobe (exon 14 N659 K), or (iii) the activation segment (exon 18 D842 V/Y or Del 845–848) [34,36]. In contrast to the wild type receptor, the mutant PDGFRα is mislocalized within the endoplasmic reticulum where it can activate JAK-STAT signaling whereas the wild type receptor localized in the plasma membrane only weakly activates STAT signaling [37].

In those patients with unresectable or metastatic GIST, treatment with imatinib is efficacious in patients with exon 12 and exon 14 mutations, but not those with exon 18 mutations (which are the most common) [38]. Although PDGFRα D842 V mutant proteins are biochemically insensitive to sunitinib [39], those patients that do not respond to imatinib generally respond to sunitinib or regorafenib as an effective second-line treatment regardless of *PDGFRA* or *KIT* mutational status [40]. Owing to the universal development of resistance to these drugs in patients with PDGFRα- or Kit-driven GIST, other small molecule antagonists are being evaluated for the treatment of drug resistant neoplasms including BLU-285, which is a potent antagonist of the *PDGFRA* D842 V activation segment mutation [41,42]. Besides DFSP and GIST, dysregulation of the PDGF/PDGFR pathways has been observed in other sarcomas [4]. Thus, co-expression of PDGF ligands and receptors has been documented in soft tissue sarcomas, osteosarcomas, synovial sarcomas, and arterial intimal sarcomas. Although pazopanib is FDA approved for the treatment of soft tissue sarcomas, its efficacy for the treatment of GIST has not been demonstrated. Besides sarcomas, *PDGFRA* gene amplifications have been described in a variety of neoplasms including esophageal squamous cell carcinomas and oligodendrogliomas [4].

The formation of fusion proteins as a result of translocations of *PDGFRA* and *PDGFRB* genes have been observed in a number of different leukemias [4]. As a result of these translocations, the amino-terminal segment of various proteins fuses with the intracellular portion of the PDGF receptors leading to dimerization and constitutive protein kinase activation [43]. The fusion of *PDGFRB* with the transcription factor TEL was observed in chronic monomylocytic leukemia. Other fusion partners include H4 (CCDC6/D10S170), HIP1, and rabaptin 5 [4,43]. PDGFR rearrangements have been observed in patients harboring different neoplasms including atypical chronic myelogenous leukemia, chronic eosinophilic leukemia, chronic myelomonocytic leukemia, and juvenile chronic myelomonocytic leukemia [43]. The most common PDGFR rearrangement is FIP1L1-PDGFRα; it is found in 10–20% of patients with idiopathic hypereosinophilia and in some cases of systemic mastocytosis. Patients with chronic myelomonocytic leukemia and chronic eosinophilic leukemia have been successfully treated with imatinib. Following the development of resistance owing to mutations in the protein kinase domain, treatment with second-line inhibitors such as nilotinib and sorafenib has been effective.

Although PDGFRβ is over expressed in both primary and metastatic prostate cancers, Nordby et al. reported that PDGFRβ was expressed in the stroma, but not in the tumor cells, of patients undergoing prostatectomy [44]. They also found that PDGFRβ expression was higher in tumor stroma when compared with normal stroma. Furthermore, they reported that the risk of clinical failure more than doubled with high PDGFRβ expression. In another study, Ustach et al. found that PDGF-DD was over expressed in prostate cancer [45]. Based upon Phase I and Phase II clinical trials, Rosenberg and Mathew reported that imatinib is ineffective in the treatment of metastatic castration-resistant prostate cancer despite the findings of high PDGFRβ expression in prostate malignancies [46]. Moreover, clinical trials with dasatinib or sunitinib have not proven to be effective in the treatment of prostate cancer. Papadopoulos and Lennartsson suggest that a better understanding of PDGF signaling in prostate cancer along with the development of new therapeutic approaches is warranted [47].

Although PDGFRs are not expressed in normal epithelial cells, there are a few examples of their expression in neoplastic epithelial cells. For example, PDGFRα and PDGFRβ over expression has been observed in some cases of hepatocellular carcinomas and such expression is correlated with a poor prognosis [4]. Using immunohistochemistry, Donnen et al. reported that a significant fraction of NSCLC cells as well as stromal cells express both PDGFRα and PDGFRβ [48]. They also found that both tumor cells and stroma expressed all five forms of PDGF. Moreover, aberrant expression of PDGFRs and PDGF ligands has also been described in lung [49], breast, colorectal, stomach, and uterine cancers [4].

Early studies on surgical specimens showed that human glioblastomas co-express PDGF-AA and PDGFRα *in vivo*, providing indirect evidence that an autocrine stimulation of the PDGF receptor pathway is a driver of tumor growth [50]. Amplification, truncations, deletions, and various rearrangements of *PDGFRA* have been described in human brain tumors [4]. Amplification of the *PDGFRA* gene has been described in 5–10% of glioblastoma cases [4]. High expression levels of the receptor enable the cells to respond to very low levels of growth factor. See Ref. [4] for a comprehensive summary of studies linking genetic aberrations of *PDGFRA* in human brain tumors. It is unfortunate that early attempts to target PDGFR in the treatment of human glioblastomas with imatinib and dasatinib have not proven successful. In studies with a human glioblastoma (GBM) cell line, Frolov et al. demonstrated that these two drugs paradoxically increased cell invasion following activation of p130Cas and focal adhesion kinase signaling [51]. Similarly, sunitinib was ineffective in the treatment of children with recurrent brain tumors. The mechanisms for the primary resistance of glioblastoma to PDGF and other receptor antagonists are unclear. However, owing to the severity of this disease, additional work in targeting PDGF/PDGFR and other signaling pathways is warranted owing to the ineffectiveness of surgery, chemotherapy, and radiation in the treatment of this illness. Prabhu et al. have reviewed the efficacy of small molecule antagonists of EGFR, Kit, PDGFR, VEGFR, topoisomerase, and tubulin that are in clinical trials for the treatment of gliomas [52].

The incidence of tumors that result chiefly from the activation of the PDGF/PDGFR pathways is small. Although there are several examples where both the receptors and PDGF ligands are expressed in cancerous cells, in most cases PDGF expression and action appear to involve the tumor stroma. Owing to the importance of the tumor microenvironment and the pivotal role that PDGF signaling plays in establishing and maintaining that microenvironment, the continued development of PDGFR antagonists represents a fruitful approach. Thus, future research on the efficacy of treating epithelial tumors with PDGF/PDGFR antagonists and whether this may synergize with other treatments targeting the tumor cells is an important question. If such synergisms are found, then the tasks of identifying the appropriate combinations and appropriate order of drug administration will need to be addressed. More than ten PDGFRα/β multikinase antagonists have been approved by the FDA for the treatment of several neoplastic diseases and interstitial pulmonary fibrosis (www.brimr.org/PKI/PKIs.htm). All of these drugs are multikinase inhibitors so that it is not known to what degree their therapeutic effects are related to PDGFR inhibition. The mode of interaction of these drugs with PDGFRα will be considered in a later section.

Overactivity of the PDGF/PDGFR pathways has been observed in a number of nonneoplastic disorders [53]. Such signaling pathways are normally operative in mesenchymal cells such as fibroblasts, smooth muscle cell, and pericytes. Pericytes are contractile cells that participate in angiogenesis and envelope the endothelial cells that line the arterioles, capillaries, and venules throughout the body [54]. Moreover, pericytes within the brain help to sustain the blood brain barrier [53]. Excessive activity of the PDGF/PDGFR pathways is linked to several fibrotic conditions including those of the bone marrow, kidney, lung, and liver as well as systemic sclerosis [4]. These pathways are also implicated in the pathogenesis of several vascular diseases including atherosclerosis, pulmonary artery hypertension, cerebral vasospasm, and assorted retinopathies.

3. Properties of the PDGFRα/β protein-tyrosine kinase domains

3.1. Primary, secondary, and tertiary structures of PDGFRα/β catalytic domains

The catalytic domain of PDGFRα consists of 362 amino acid residues and that of PDGFRβ consists of 363 residues while the average protein kinase domain contains about 275 residues. The larger size of the PDGFR family is due to the inclusion of a kinase insert domain (KID) of about 100 residues. The stoichiometry of the PDGFR protein kinase reaction is given by the following chemical equation:

$$\mathrm{MgATP^{1-} + protein\text{-}tyrosine\text{–}O:H \rightarrow protein\text{-}tyrosine\text{–}O:PO_3^{2-} + MgADP + H^+}$$

We see that the phosphoryl (PO_3^{2-}) moiety and not the phosphate (OPO_3^{2-}) group is transferred from ATP to the protein-tyrosine substrate.

Based upon the primary structures of about five dozen protein-serine/threonine and protein-tyrosine kinases, Hanks and Hunter divided protein kinases into 12 domains (I-VIA, VIB-XI) [55]. Domain I of protein kinases including PDGFRα/β contains a glycine-rich loop (GRL) with a GxGxΦG signature (^{600}GSGAFG^{605}/^{609}GSGAFG^{614}), where Φ refers to a hydrophobic residue and is phenylalanine in the case of PDGFRα/β. The glycine-rich loop links the β1- and β2-strands and overlays the ATP-binding site. Because of its role in both ATP binding and ADP release, the glycine-rich loop must be flexible and protein segments containing multiple glycines possess this property. Domain II of PDGFRα/β contains a conserved Ala-Xxx-Lys (^{625}AVK^{627}/^{632}AVK^{634}) sequence in the β3-strand and domain III contains a conserved glutamate (E644/E651) in the αC-helix that forms an electrostatic bond with the conserved β3-lysine in some dormant protein kinase conformations (Fig. 4A) and all active protein kinase conformations (Fig. 4D). Domain VIB of PDGFRα/β contains a conserved HRD sequence, which forms part of the catalytic loop (^{816}HRDLAARN^{823}/^{824}HRDLAARN^{831}). PDGFRα/β domain VII contains an ^{836}DFG^{838}/^{844}DFG^{846} signature and domain VIII contains an ^{863}APE^{865}/^{871}APE^{873} sequence, which together represent the beginning and end of the PDGFRα/β activation segments. The activation segment exhibits different conformations in the active and

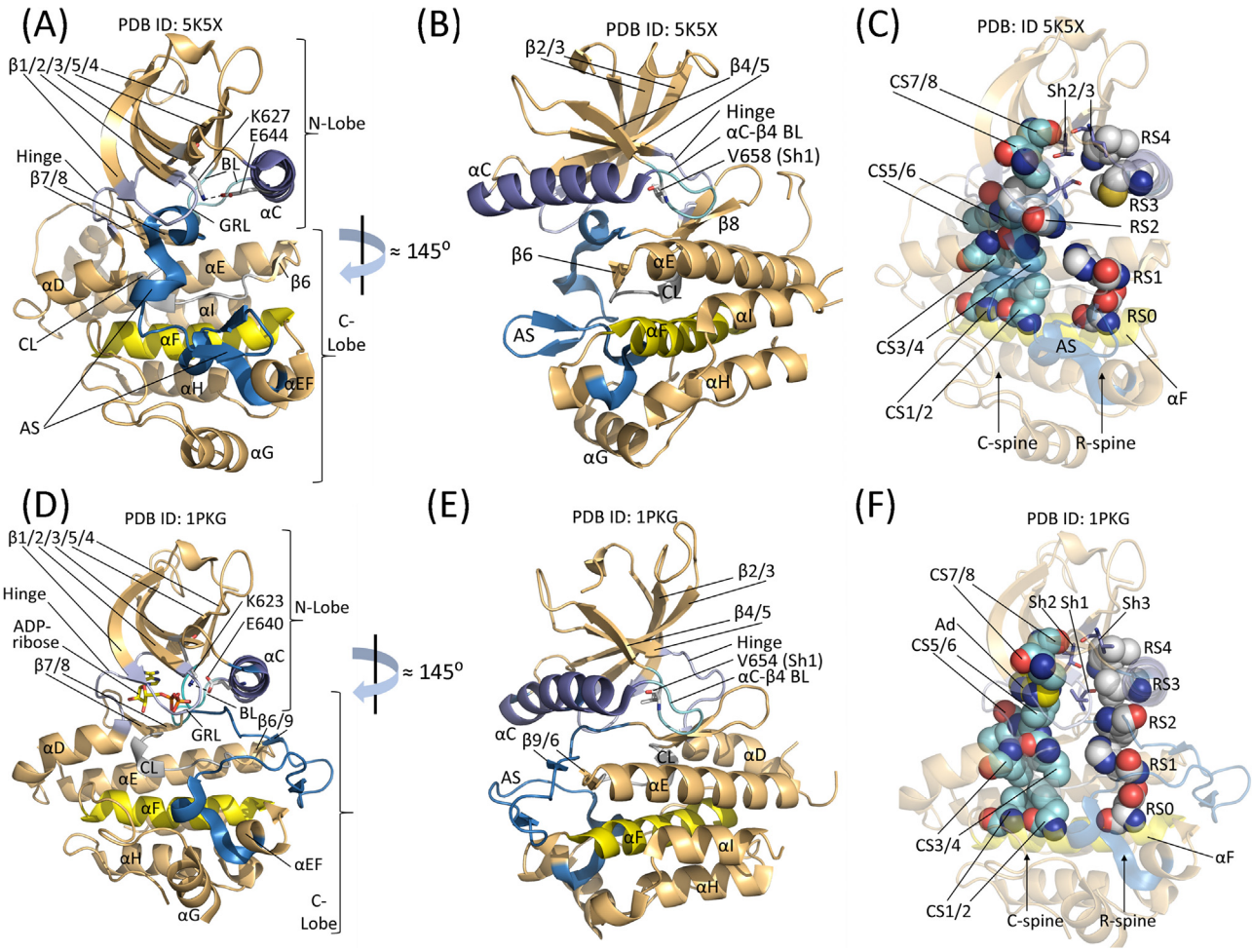

Fig. 4. Overview of the structures of dormant PDGFRα (A and B), the PDGFRα spine and shell residues (C), active Kit (D and E), and the Kit spine and shell residues (F). Ad, adenine; AS, activation segment; BL, αC-β4 back loop; CL, catalytic loop; CS, catalytic spine; GRL, glycine-rich loop; RS, regulatory spine; Sh, shell.

dormant states. Domains IX–XI form the αF–αI helices. The X-ray structure of the catalytic subunit of protein kinase A (PKA) provided a valuable framework for formulating the roles of the 12 Hanks domains and the structure has clarified our views on the underlying biochemistry of the entire protein kinase superfamily (PDB ID: 2CPK) [56,57]. All protein kinases have a small amino-terminal and a large carboxyterminal lobe [58,59]. PDGFRα has this characteristic structure; although there is no structure of PDGFRβ in the public domain, it would be unprecedented if it failed to adhere to this principle. The small lobe contains five conserved β-strands (β1–5) and an important regulatory αC-helix and the large lobe of active enzymes contains four conserved β-strands (β6–β9) along with seven helices (αD–αI and αEF) (Fig. 4A). Of the hundreds of protein kinase structures that have been determined, all of them resemble the original protein kinase fold as first described for PKA [56,57,59].

All functional protein kinases contain a K/E/D/D (Lys/Glu/Asp/Asp) amino acid signature that plays an important role in protein kinase catalysis (Table 3) [56]. The lysine and glutamate occur within the small lobe and the two aspartate residues occur within the large lobe. Although ATP binds in the cleft between these lobes, there is more extensive interaction with the small lobe. Comprehensive structural studies indicate that a salt bridge between the β3-lysine and the αC-glutamate is a prerequisite for the formation of an active protein kinase, which corresponds to an "αC_{in}" configuration as shown for active Kit (Fig. 4D). These residues in many dormant enzymes fail to form this salt bridge and thereby form an inactive "αC_{out}" structure (See Refs. [59,60] for details). The αC_{in} structure is necessary, but not sufficient, for the expression of catalytic activity. Although the β3-K627 and αC-E644 of PDGFRα form a salt bridge (Fig. 4A), molecular measurements indicate that this corresponds to an αC-dilated conformation as described later. Moreover, the activation segment of dormant PDGFRα is in a closed conformation that blocks ATP and protein substrate binding. The proximal portion of the closed activation segment of PDGFRα contains an inhibitory α-helix. The activating D842 V mutation of GIST [34] occurs within this segment and this mutation may destabilize the dormant enzyme leading to kinase activation.

The large lobe contains catalytic loop residues that play an essential role during the reaction cycle. Furthermore, two Mg^{2+} ions have been demonstrated to participate during each catalytic cycle of several protein kinases [61] and they are presumably required for the proper functioning of PDGFRα/β. Of the two PDGFRα X-ray structures in the public domain, neither contains ATP, a protein/peptide substrate, nor Mg^{2+}. Thus, we have chosen an active form of the related Kit receptor protein-tyrosine kinase for comparative purposes. By inference, PDGFRα/β D836/844 (the DFG-D and the second D of K/E/D/D) binds to $Mg^{2+}(1)$, which in turn binds to the β- and γ-phosphates of ATP. In the active conformation, the DFG-D is directed inward toward the active site as depicted for Kit (Fig. 5A). In contrast, the DFG-D of PDGFRα is pointed outward producing an inactive DFG-D_{out} structure (Fig. 5B).

Table 3
Important residues in human PDGFRα/β[a].

	PDGFRα	PDGFRβ	Inferred function	Hanks no.
UniProtKB accession no.	P16234	P09619		
No. of residues	1089	1106		
Molecular Wt (kDa)[b]	122.7	124.0		
Signal sequence	1–23	1–32		
Extracellular domain	24–528	33–532		
Ig-D1	24–113	33–120		
Ig-D2	117–201	129–210	PDGF binding	
Ig-D3	202–306	214–309	PDGF binding	
Ig-D4	319–410	331–403	Promotes receptor dimer formation	
Ig-D5	414–517	416–524	Promotes receptor dimer formation	
TM segment	529–549	533–553	Links extracellular and intracellular domains and mediates receptor dimer formation	
JM segment	550–592	554–600	Regulatory role	
JM segment tyrosine phosphorylation sites	572, 574	579, 581	Signal transduction	
Protein kinase domain	593–954	600–962	Catalyzes substrate transphosphorylation	
Glycine-rich loop	^{600}GSGAFG^{605}	^{609}GSGAFG^{614}	Anchors ATP β-phosphate	I
β3-K of K/E/D/D	K627	K634	Forms salt bridges with ATP α- and β-phosphates and with αC-E	II
αC-E, E of K/E/D/D	E644	E651	Forms salt bridges with β3-K	III
Hinge residues	^{675}EYCFYG^{680}	^{682}EYCRYG^{687}	Connects N- and C-lobes and hydrogen bonds with the ATP adenine	V
Kinase insert domain	691–789	698–797	Signal transduction	
Kinase domain tyrosine phosphorylation sites	720, 731, 742, 754, 762, 768, 849	716, 740, 751, 763, 771, 775, 857, 934	Interact with a variety of docking proteins that mediate intracellular signaling	
Catalytic loop, HRDLAARN	816–823	824–831	Plays both structural and catalytic functions	VIb
Catalytic loop HRD-D, First D of K/E/D/D	D818	D826	Catalytic base (abstracts protein substrate proton)	VIb
Catalytic loop Asn, HRDLAARN	N823	N831	Chelates Mg^{2+}(2)	VIb
AS DFG-D, Second D of K/E/D/D	D836	D844	Chelates Mg^{2+}(1)	VII
AS	836–865	844–873	Positions protein substrate	VII-VIII
AS tyrosine phosphorylation sites	Y849	Y857	Stabilizes the AS after phosphorylation	VIII
End of AS	^{863}APE^{865}	^{871}APE^{873}	Interacts with the αHI loop and stabilizes the AS	VIII
C-terminal tail	866–1089	874–1106	Signal transduction	None
C-terminal tail tyrosine phosphorylation sites	988, 1018	1009, 1021	Mediate intracellular signaling	None

[a] AS, activation segment; JM, juxtamembrane; TM, transmembrane.
[b] Molecular weight of the unprocessed and nonglycosylated precursor.

Vijayan et al. examined the structures of hundreds of protein kinases and they divided the DFG-D_{out} structures into (i) classical and (ii) nonclassical groups [62]. This distinction was made because of variations in the locations of the activation segment aspartate and phenylalanine in the eukaryotic kinome. They formulated two measurements that characterized these groups and labeled them D1 and D2. D1 is the distance between the αC atom of the DFG-F and the asparagine at the end of the catalytic loop and D2 is the distance between the αC atom of the DFG-F and the αC-E residue. The enzyme has a classical DFG-D_{out} structure if D1 is less than 7.2 Å and D2 is greater than 9 Å. In the case of the structure of PDGFRα (PDB:ID 5K5X), D1 equals 5.8 Å and D2 equals 11.8 Å; accordingly, this falls into the classical DFG-D_{out} category. The structure of Kit shows D1 and D2 values of 9.0 Å and 4.9 Å that are not in line with a classical DFG-D_{out}, but are consistent with the DFG-D_{in} classification (Fig. 5A and B). The relative location of the αC-helix and the β3-strand is an important structural parameter. The salt bridge between β3-K and αC-E is broken in the inactive αC_{out} configuration. However, Vijayan et al. reported that this ion-pair interaction occurs in ≈ 90% of classical DFG-D_{out} structures; they refer to these as αC-dilated structures to distinguish them from αC_{in} structures [62]. They measured the distance from the α-carbon atoms of the αC-E and the DFG-D, which we will call D3, and found that a D3 value of less than 9 Å corresponds to αC_{in} while those with a D3 value greater than 10.5 Å corresponds to αC_{out}. The αC-dilated structure has a D3 value greater than 9 Å and less than 10.5 Å (Fig. 5A and B).

The exocyclic 6-amino nitrogen of ATP would characteristically form a hydrogen bond with the carbonyl backbone residue of the first PDGFRα hinge residue (E675) that connects the amino-terminal and carboxyterminal lobes of the protein kinase domain and the *N*1 nitrogen of the adenine base would form a second hydrogen bond with the N—H group of the third hinge residue (C677). The adenine binding pocket is located adjacent to these hinge residues. As noted later, most small-molecule steady-state ATP competitive antagonists of protein kinases including the PDGF receptors make hydrogen bonds with the backbone residues of the connecting hinge.

The protein kinase activation segment, which is typically 20–30 residues in length, plays an important role in the catalytic cycle [63]. The origin of the segment is located near the amino-terminus of the αC-helix and the conserved HRD of the catalytic loop. These components are linked by hydrophobic interactions. As with most protein kinases, phosphorylation of a residue within the activation segment converts a dormant to a catalytically active enzyme [64,65]. Fantl et al. reported that phosphorylation of tyrosine 856 within the activation segment of murine PDGFRβ and Kazlauskas et al. reported that phosphorylation of tyrosine 857 in human PDGFRβ are associated with increased enzyme activity [66,67]. Lemmon and Schlessinger reviewed the mechanisms for stimulating receptor protein-tyrosine kinases and this process involves the growth factor-induced formation of receptor dimers and subsequent protein kinase activation or it involves the growth factor-induced activation of preformed dimers [68]. In the case of PDGFRβ, growth factors promote dimerization and subsequent activation. During activation, one member of the dimer pair catalyzes (i) the phosphorylation of one or more activation segment tyrosine residues

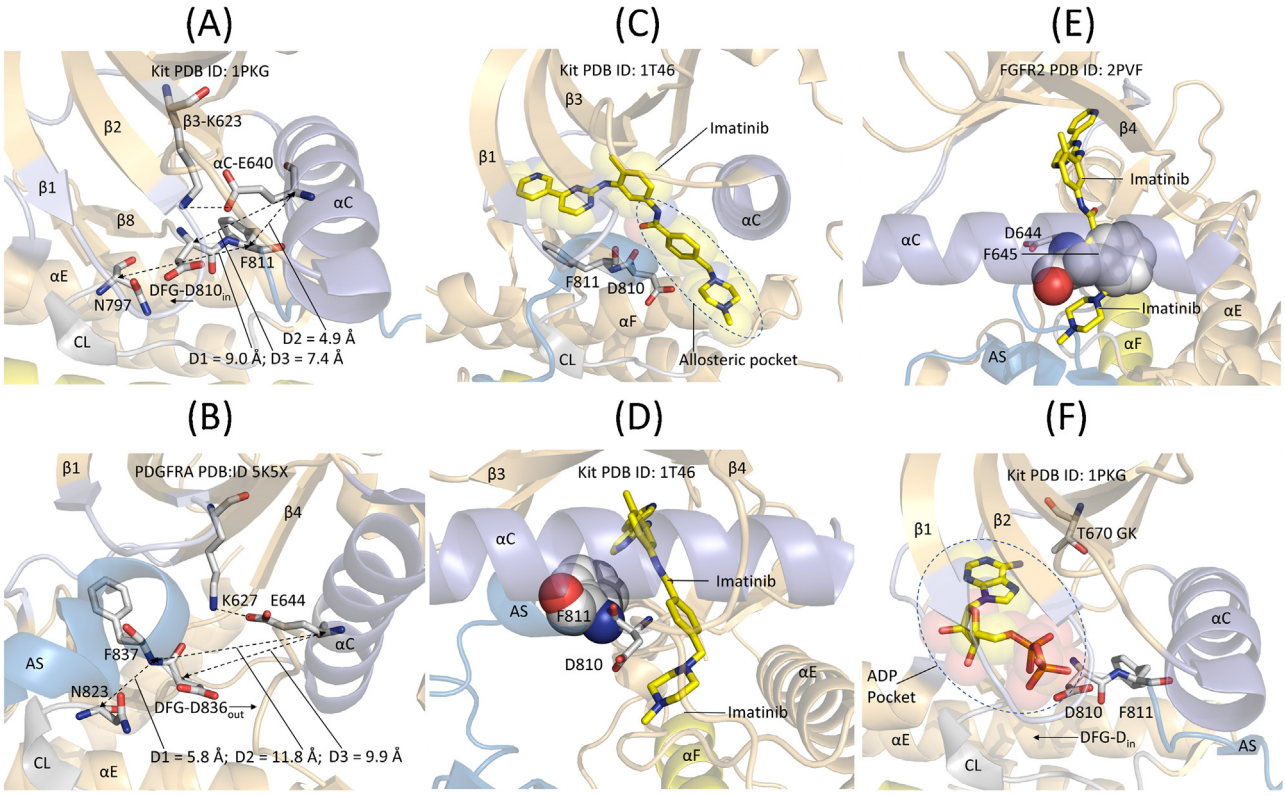

Fig. 5. (A) Kit DGF-D_{in}. (B) PDGFRα DFG-D_{out}. The dashed lines indicate polar bonds and the dashed double arrows in A and B indicate distance measurements (D1, D2, D3). (C) Kit allosteric pocket. (D) Imatinib binding to Kit DFG-D_{out} conformation. (E) Inability of imatinib to bind to the FGFR2 DFG-D_{in} conformation owing to steric hindrance with DFG-F. This was prepared by superposing imatinib bound to Kit (PDB ID: 1T46) onto active FGFR2. (F) Kit ATP/ADP binding pocket. AS, activation segment; CL, catalytic loop.

and (ii) other residues of the receptor partner (both in *trans*). Such phosphorylation reactions outside of the activation segment create docking sites for signal transduction proteins. In addition to the activation segment, PDGFRs are autoinhibited by the JM segment and by the carboxyterminal tail. Baxter et al. reported that phosphorylation of Y579 and Y581 within the JM segment contributes to PDGFRβ activation and these phosphorylation reactions occur prior to activation segment Y857 phosphorylation [69].

The PDGFRα HRDLAARN catalytic loop aspartate (D818), which is the first D of the K/E/D/D signature, functions as a base that abstracts a proton from the protein-tyrosine substrate thereby facilitating the nucleophilic attack onto the γ-phosphorus atom of ATP (Fig. 6) [70]. The activation segment, when it is in its open and active conformation, positions the protein substrate. By similarity, β3-K627 forms salt bridges with αC-E644 and the α- and β-phosphates of ATP. Moreover, Mg^{2+}(1) binds to the β- and γ-phosphates while Mg^{2+}(2) and β3-K627 bind to the α- and β-phosphates of ATP thereby facilitating catalysis. The catalytic segment AAR sequence occurs in many receptor protein-tyrosine kinases such as PDGFRα/β and EGFR while RAA occurs in many non-receptor protein-tyrosine kinases including Src [55]. However, the functional significance of these differences in the catalytic segment is unclear.

Under physiological conditions, the activity of protein kinases and downstream signaling events are stringently regulated. Besides activation, binding of PDGF to its receptors leads to receptor mediated endocytosis and degradation of the ligand-receptor complex in endosomes; both the ligand and receptor are degraded following fusion with lysosomes [71]. Moreover, activated receptors undergo ubiquitylation, a process that targets them for degradation in proteasomes. The internalization rate of the PDGFRβ is dependent on its protein kinase activity and Tyr579 phosphorylation in the JM domain modulates internalization. Phosphorylated Tyr579 binds Src protein-tyrosine kinase, but it is unclear if it is involved in the internalization process. PI–3K is another signal transduction molecule that has been implicated in PDGF receptor internalization; a mutant receptor lacking the PI3–kinase tyrosine residues

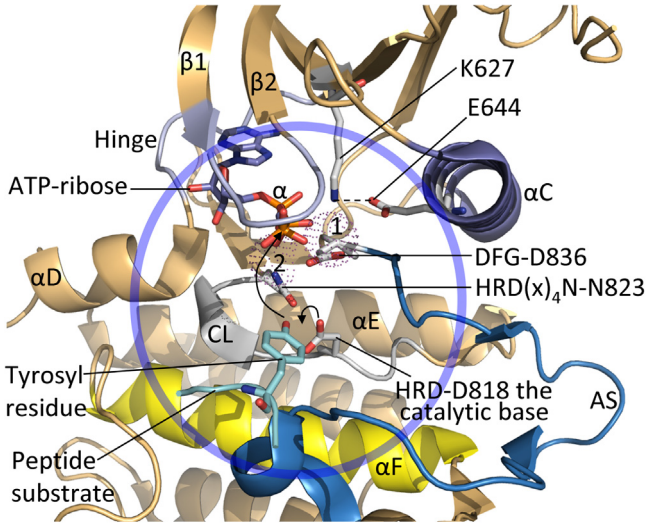

Fig. 6. Inferred mechanism of the PDGFRα catalyzed protein kinase reaction. D818 abstracts a proton from the tyrosyl substrate allowing for its nucleophilic attack onto the γ-phosphate of ATP. The chemistry occurs within the blue circle. 1 and 2 label the two Mg^{2+} ions shown as dots. AS, activation segment; CL, catalytic loop. The figure was prepared from FGFR2 (PDB ID: 2PVF), but the residue numbers correspond to those of PDGFRα.

required for binding was defective in ligand-induced internalization.

Besides receptor endocytosis, protein-tyrosine phosphatase activity directed against the receptor and downstream proteins exert negative effects on PDGFR signaling [71]. The superfamily of protein phosphatases is large and consists of about 100 gene products [72]. The largest phosphatase family employs a cysteine thiol as a nucleophile within the active site. Members of this family are able to catalyze the hydrolysis of pTyr, pSer, and pThr residues along with different phosphatidylinositol derivatives; many of these enzymes are dual specificity phosphatases that act on both phosphorylated protein-tyrosine and protein-serine/threonine substrates. Aspartate-based and histidine-based phosphatase families are much smaller and they target pTyr, pSer, and pThr. Shp-2 is an enzyme that can mediate the hydrolysis of PDGF receptor phosphotyrosines; this enzyme is a classical cysteine-based nonreceptor, or cytosolic, protein tyrosine phosphatase. In contrast, receptor protein-phosphatases are plasma membrane-associated enzymes. Of the 125 members of the human PTPome, only about 40 are able to catalyze the dephosphorylation of pTyr exclusively.

3.2. The hydrophobic spines of PDGFRα/β and Kit

Kornev et al. analyzed the structures of 23 protein kinases and they established the role of several indispensable residues by a local spatial pattern alignment algorithm [73,74]. They classified four hydrophobic residues as a regulatory or R-spine and eight hydrophobic residues as a catalytic or C-spine. The R-spine and C-spine contain amino acid residues from both the small and large lobes. The R-spine contains one residue from the activation segment and another from the regulatory αC-helix, both of which are major components that may toggle between more active and less active enzyme states. The lower portion of the R-spine within the carboxyterminal lobe anchors the activation segment and catalytic loops in an active state and the C-spine tethers ATP within the active site cleft thus enabling catalysis. Moreover, the proper alignment of both spines is necessary for the production of an active enzyme as described for ALK, the cyclin-dependent protein kinases, EGFR, ERK1/2, the Janus kinases, MEK1/2, RET, ROS1, Src, and VEGFR1/2/3 [29,31,61,75–82].

Going from the base to the apex, the standard protein kinase R-spine consists of the catalytic loop HRD-H, the activation loop DFG-F, an amino acid four residues C-terminal to the conserved αC-glutamate, and an amino acid at the proximal end of the β4-strand [73,74]. The backbone N—H of the HRD-His forms a hydrogen bond with an invariant aspartate carboxyl group within the αF-helix. Again, going from the base to the apex of the R-spine, Meharena et al. named the R-spine residues RS0, RS1, RS2, RS3, and RS4 [83]. The R-spine of inactive enzymes such as PDGFRα with DFG-D_{out} is nonlinear and broken with a displaced RS2 residue (Fig. 4C) (See Refs. [59,84] for details). We surmise that the R-spine of active PDGFRα/β with DFG-D_{in} is linear like that of the active Kit (Fig. 4F). The C-spine of protein kinases contains residues from both the small and large lobes; this spine is completed by the adenine base of ATP (Fig. 4F) [74]. The two residues of the small N-lobe that interact with the ATP adenine include a conserved valine at the beginning of the β2-strand (CS7) and an alanine residue from the signature AxK of the β3-strand (CS8). Moreover, a hydrophobic residue from the β7-strand (CS6) on the floor of the ATP pocket interacts with the ATP adenine. Almost all, if not all, ATP-competitive protein kinase inhibitors interact with CS6. The CS6 residue occurs between two hydrophobic residues (CS4 and CS5) that interact with the CS3 residue near the beginning of the αD-helix of the large C-terminal lobe. CS5/6/4 immediately follow the catalytic loop asparagine (HRDxxxxN) so that these residues can readily be determined from the primary structure. Finally, the hydrophobic CS3 and CS4 residues interact with CS1 and CS2 of the hydrophobic αF-helix thereby forming a completed catalytic spine (Fig. 4C and F) [74]. Note that the αF-helix, which spans the entire large C-terminal lobe, anchors both spines. Moreover, both the R-spine and C-spine play an indispensable role in anchoring the protein kinase catalytic residues in an active state. CS7 and CS8 in the small amino-terminal lobe make up the “ceiling” of the adenine-binding pocket while CS6 in the large carboxyterminal lobe makes up the “floor” of the binding pocket. Furthermore, CS5/6/4 make up the β7-strand of the protein kinase domain.

Based upon site-directed mutagenesis studies, Meharena et al. characterized three shell (Sh) residues in the PKA catalytic subunit that serve as ligaments to strengthen the R-spine, which they named Sh1, Sh2, and Sh3 [83]. Their Sh2 residue corresponds to the canonical gatekeeper residue. The gatekeeper residue plays an important role in controlling access to the back cleft [85,86] within hydrophobic pocket II (HPII) [86,87]. The Sh1 residue occurs in the αC-β4 back loop (Fig. 4B and E). In contrast to the identification of the APE, DFG, or HRD signatures, which is based upon their primary structures [55], the spines were identified by their spatial locations in active or dormant protein kinases [73,74]. Table 4 provides a summary of the spine and shell residues of PDGFRα/β, Kit, and PKA. As noted later, small molecule protein kinase antagonists often interact with residues that constitute the C-spine as well as the R-spine and shell residues [84].

Although the PDGFRα X-ray structure shows that the β3-K627 and the αC-E644 form a salt bridge (αC-dilated), the structure is that of a dormant protein kinase with the DFG-D_{out} configuration. As a consequence, the R-spine is broken. The Kit X-ray structure is that of an active protein kinase; the DFG-D is pointed inward toward the active site, the configuration of the αC-helix is in its active αC_{in} configuration with E640 forming a hydrogen bond with the β3-K623, and the catalytic and regulatory spines are linear and are neither bent nor broken (Fig. 4D–F).

4. FDA-approved PDGFR inhibitors

4.1. Classification of protein kinase-drug complexes

Dar and Shokat defined three classes of low molecular weight protein kinase inhibitors: types I, II, and III [88]. The type I group binds within the ATP pocket of an active conformation of the kinase domain; the type II inhibitors bind to an inactive conformation of the enzyme with DFG-D_{out} while the type III inhibitors bind at an allosteric site, which is defined as a site that is separate from the active site [89] and outside of the ATP-binding pocket. Zuccotto defined type I½ inhibitors as drugs that bind to an inactive protein kinase with the DFG-D directed inward (DFG-D_{in}) toward the active site (in contrast to the DFG-D_{out} structure) [90]. The inactive enzyme may have the αC_{out} conformation or it may exhibit abnormalities in the activation segment or the regulatory spine. In a later paper, Gavrin and Saiah divided allosteric inhibitors into two classes: III and IV [91]. Accordingly, type III inhibitors bind within the cleft between the amino-terminal and carboxyterminal lobes contiguous with, but independent of, the ATP binding site while type IV allosteric inhibitors bind elsewhere. Lamba and Gosh defined bivalent inhibitors as those ligands that span two distinct parts of the protein kinase domain as type V inhibitors [92]. For example, an antagonist that binds to the ATP-pocket and the peptide substrate site would be classified as a type V inhibitor. To complete this classification, we classified small molecules that form covalent adducts with the target enzyme as type VI inhibitors [84]. Afatinib is a type VI covalent inhibitor of EGFR that is FDA approved for the treatment of NSCLC. Mechanistically, this drug binds initially

Table 4
Spine and shell residues of human PDGFRα/β, Kit, and murine PKA.

	Symbol	KLIFS No.[a]	PDGFRα	PDGFRβ	Kit	PKA[b]
Regulatory spine						
β4-strand (N-lobe)	RS4	38	L660	L667	L656	L106
C-helix (N-lobe)	RS3	28	M648	M655	L644	L95
Activation loop F of DFG (C-lobe)	RS2	82	F837	F845	F811	F185
Catalytic loop His/Tyr (C-lobe)	RS1	68	H816	H824	H790	Y164
F-helix (C-lobe)	RS0	None	D877	D885	D851	D220
R-shell						
Two residues upstream from the gatekeeper	Sh3	43	I672	I679	V668	M118
Gatekeeper, end of β5-strand	Sh2	45	T674	T681	T670	M120
αC-β4 loop	Sh1	36	V658	V655	V654	V104
Catalytic spine						
β3-AxK motif (N-lobe)	CS8	15	A625	A632	A621	A70
β2-strand (N-lobe)	CS7	11	V607	V614	V603	V57
β7-strand (C-lobe)	CS6	77	L825	L833	L799	L173
β7-strand (C-lobe)	CS5	78	L826	I834	I798	I174
β7-strand (C-lobe)	CS4	76	V824	V832	I798	L172
D-helix (C-lobe)	CS3	53	L682	L689	L678	M128
F-helix (C-lobe)	CS2	None	I888	I896	L862	L227
F-helix (C-lobe)	CS1	None	L884	L892	F858	M231

[a] KLIFS (kinase–ligand interaction fingerprint and structure) from Ref. [97].
[b] From Refs. [73,74,83].

to an active EGFR conformation (like a type I inhibitor) and then the EGFR sulfhydryl group of C797 attacks the drug to form a covalent adduct [84].

We previously divided the type I½ and type II inhibitors into A and B subtypes [84]. Ponatinib is a type II inhibitor of Kit (PDB ID: 1T46). This drug binds to the protein kinase domain with the DFG-D_{out} configuration and courses into the back cleft [84] and we classified drugs that extend into the back cleft as type A inhibitors. On the other hand, we classified drugs such as sunitinib that bind to the Kit DFG-D_{out} conformation, but not extending into the back cleft, as type IIB inhibitors (PDB ID: 3G0E). Based on limited data [84], the potential consequence of this distinction is that type A inhibitors bind to their target kinase with a long residence time when compared with type B antagonists.

Type II inhibitors bind to their target with the DFG-D pointing away from the active site and they are usually the easiest to identify. As a consequence, the DFG-D and DFG-F switch positions and the movement of the phenylalanine creates a large allosteric pocket that interacts with portions of type II inhibitors such as imatinib (Fig. 5C). Type I and I½ inhibitors such as dasatinib, lenvatinib, and sunitinib do not interact with this hydrophobic pocket, but in many instances, they are able to bind to the DFG-D_{out} configuration. As a consequence of the shift of DFG-D and DFG-F, type II inhibitors such as imatinib are able to bind to the DFG-D_{out} conformation (Fig. 5D). In contrast, imatinib is unable to bind to the DFG-D_{in} conformation owing to the steric hindrance of the drug with DFG-F (Fig. 5E). Imatinib and phenylalanine cannot simultaneously occupy the same space. The ATP/ADP binding site occurs within the front pocket and does not extend beyond the gatekeeper residue (Fig. 5F). In the next section, we will see that the PDFGRα antagonists studied in this paper are type II inhibitors.

4.2. Structures of PDGFRα-drug complexes

Axitinib (*N*-Methyl-2-((3-(2-(pyridin-2-yl)vinyl)-1H-indazol-6-yl)thio)benzamide) (Fig. 7A) is a second generation orally effective PDGFRα/β and VEGFR1/2/3 antagonist that was FDA approved for the treatment of renal cell carcinomas in 2012. Both PDGF and VEGF receptors participate in angiogenesis and the observed therapeutic response may be related to the inhibition of both of these families of enzymes [54,93,94]. Axitinib is about eight-fold more potent against the VEGF receptor family than the PDGF receptor family with low nM IC_{50} values. Although there are no structural data of axitinib bound to PDGFRα/β, we were able to generate a satisfactory pose of the drug binding to human PDGFRα using the Schrödinger induced-fit docking protocol (2016-1 release) [95] with 5GRN (PDB: ID) as a template. The term pose refers to the description of the position and orientation of the drug relative to its target [96]. The resulting structure indicates that the *N*1 N—H group of the indazole forms a hydrogen bond with the carbonyl group of E675 (the first hinge residue) and *N*2 forms a hydrogen bond with the N—H group of C677 (the third hinge residue). This interaction mimics that of ATP with the hinge residues of the protein kinase family [59]. The benzamide carbonyl group hydrogen bonds with the N—H group of DFG-D836 and the benzamide N—H group forms a hydrogen bond with αC-E644 (Fig. 8A). Moreover, the β3-K627 forms a hydrogen bond with DFG-D836 (not shown). The drug interacts hydrophobically with residues near the ceiling of the adenine pocket including L599 at the end of the β1-strand, V607 at the beginning of the β2-strand, and A625 (CS8) within the β3-strand. Axitinib also interacts hydrophobically with V658 (Sh1) within the αC-β4 back loop, I672 in the β5-strand, Y676 within the hinge, L825 (CS6) within the β7-strand on the floor of the adenine binding pocket, and DFG-F837 (RS2). The pyridine ring of axitinib is directed away from the enzyme into the solvent.

Liao [87] and van Linden et al. [97] divided the cleft between the small amino-terminal and large carboxyterminal lobes into a front cleft, a gate area, and a back cleft. The back cleft and gate area make up the back pocket or hydrophobic pocket II (HPII) (Fig. 9). The front cleft includes the glycine-rich loop, the hinge, the linker connecting the hinge to the αD-helix in the large lobe, and the amino acid residues within the catalytic loop. The gate area includes amino acids within the β3-strand and the proximal activation segment including DFG. The back cleft extends to the αC-helix, the αC-β4 back loop, a section of the β4- and β5-strands, and a portion of the αE-helix.

van Linden et al. described several sub-pockets within these three regions [97]. Thus, the front cleft contains an adenine pocket (AP) contiguous with two front pockets (FP-I and FP-II**).** FP-I is located between the solvent-exposed linker connecting the hinge to the αD-helix and the DFG-motif (xDFG where x is the residue before DFG) and FP-II is located between the G-rich loop and the β3-strand. Back pocket I (BP-I) is located in the gate area between

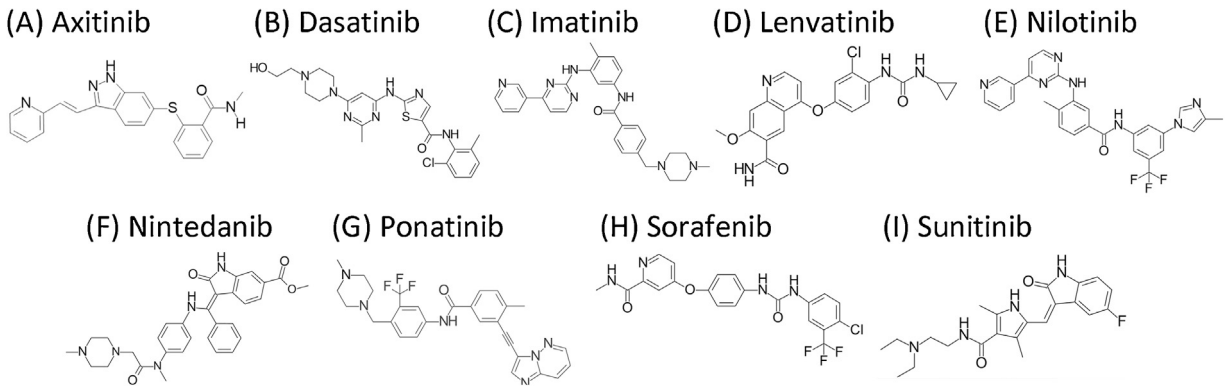

Fig. 7. Structures of selected PDGF receptor multikinase inhibitors.

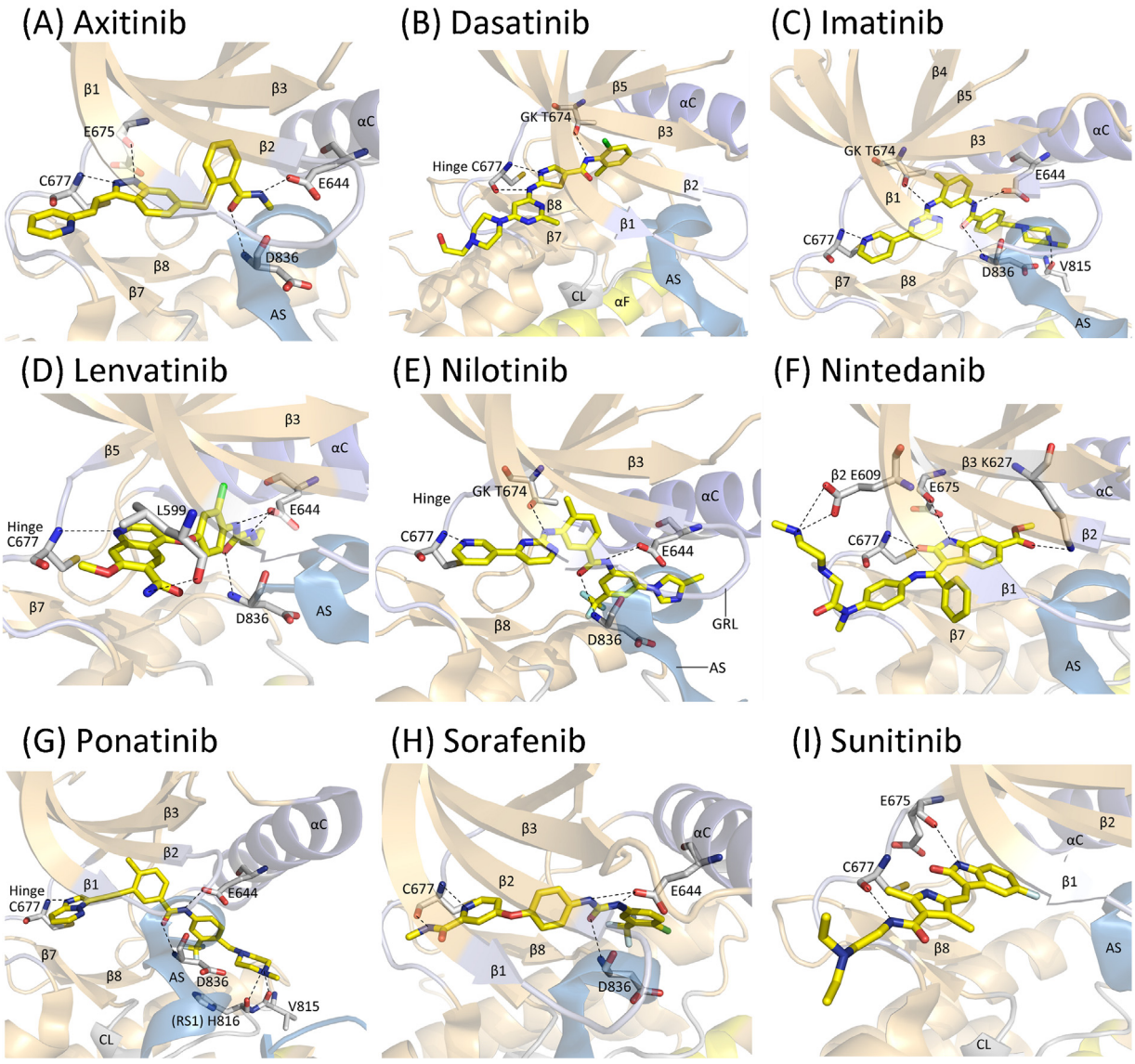

Fig. 8. Structures of PDGFRα-drug complexes. AS, activation segment; CL, catalytic loop; GK, gatekeeper. The dashed lines depict polar bonds.

residues of the xDFG-motif, the β3- and β4-strands, the conserved β3-K, and the αC-helix. BP-I can be divided into two subpockets (BP-I-A and BP-I-B). The smaller BP-I-A is located at the top of the gate area and is formed by residues of the β3- and β5-strands, β3-K, and the αC-helix. The larger BP-I-B is located in the center of the gate area allowing access to the back cleft. BP-I-A and BP-I-B exist in both DFG-D_{in} and DFG-D_{out} conformations

Extending into the back cleft, BP-II-A-in and BP-II-in occur in the DFG-D_{in} conformation. These pockets consist of the DFG-motif, the αC-helix, the back loop, and the β4- and β5-strands. Significant

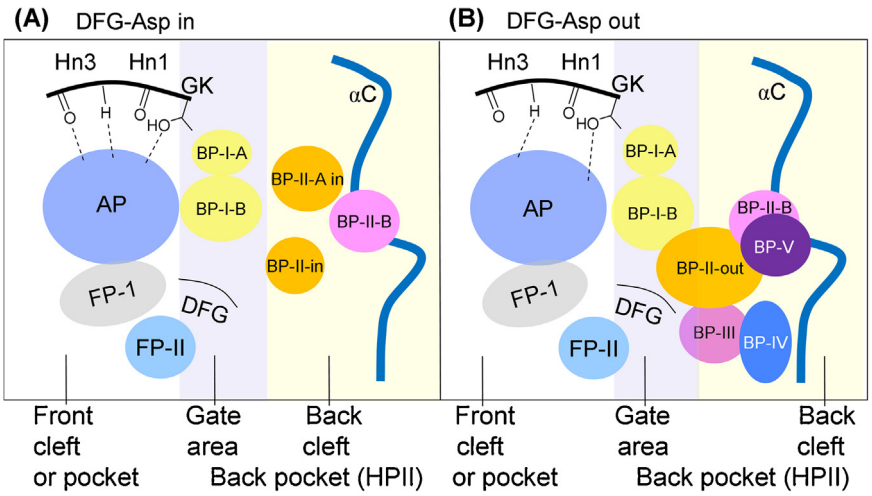

Fig. 9. Structure of the protein kinase domain drug-binding pockets. AP, adenine pocket; BP, back pocket; FP, front pocket; Hn, hinge; HPII, hydrophobic pocket II. Adapted from Refs. [87,97].

modification of BP-II-A-in and BP-II-in occurs to yield BP-II-out in the DFG-D_{out} conformation; this difference is associated with the change in the location of DFG-F. The resulting pocket is termed back pocket II-out (BP-II-out); it is positioned where the DFG-F occurs in the DFG-D_{in} conformation. BP-II-B is located between the αC-helix and β4-strand in both DFG-D_{in} and DFG-D_{out} conformations. Back pocket III (BP-III) is accessible only in the DFG-D_{out} conformation. This pocket is located on the floor of BP-II-out between the DFG-D_{out} motif, the αC-β4 back loop, the αC- and αE-helices, the β6-strand, and the conserved HRD-H residue of the catalytic loop. Two partially hydrophobic back pockets (BP-IV and BP-V) are located between the αC-helix, the DFG-D_{out} motif, the β6-strand, the catalytic loop, and the activation segment (Fig. 9). These pockets are partially solvent exposed. The computer-generated structure shows that axitinib binds to the PDGFRα front pocket, gate area, and back pocket including BP-I-B and BP-II-out. Although the αC-E644 forms a hydrogen bond with the β3-K627, the molecular structure shows that PDGFRα is in a dormant DFG-D_{out} conformation. Accordingly, axitinib is classified as a type II inhibitor of PDGFRα [84].

van Linden et al. have created a comprehensive synopsis of ligand and drug binding to more than 1200 human and mouse protein kinase catalytic domains [97]. Their KLIFS (kinase–ligand interaction fingerprint and structure) listing includes the alignment of 85 protein kinase-ligand binding-site residues; this facilitates a classification of ligands based upon their binding properties and expedites the recognition of related interactions. Moreover, these investigators created a standard amino acid residue numbering system that facilitates a comparison among all protein kinases. See Table 4 for the relationship between the KLIFS database numbering and the R-spine, Shell, and C-spine amino acid residue classification. Of practical importance, this consortium established a valuable noncommercial and searchable web site that is regularly updated and provides valuable information on protein kinase-ligand interactions (klifs.vu-compmedchem.nl/)

Dasatinib (*N*-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl]amino]-1,3-thiazole-5-carboxamide) (Fig. 7B) is a PDGFR and BCR-Abl multikinase antagonist that is FDA approved for the treatment of Philadelphia chromosome positive acute lymphoblastic and chronic myelogenous leukemias (Table 5). Chronic myelogenous leukemia is characterized by the activation of the Abl kinase following the translocation of its gene to the breakpoint cluster region (BCR) with the concomitant formation of the Philadelphia chromosome that encodes a constitutively active BCR-Abl protein kinase chimera [98]. The blockade of a single enzyme by imatinib and by second generation antagonists such as dasatinib is thus efficacious in the treatment of this illness. Essentially all other cancers arise from the dysregulation of many signaling pathways. The remarkable success in the treatment of this leukemia by an inhibitor of the BCR-Abl kinase is due to the unique pathogenesis involving a single biochemical defect. It is notable that this distinctive characteristic is absent from basically all other forms of malignancy and the development of inhibitors of single targets is generally less effective in treating other cancers [59].

Dasatinib was initially developed as a BCR-Abl antagonist and was later shown to inhibit PDGFRβ [99,100]. Its therapeutic effect may be related to the inhibition of Src family kinases, Kit, and PDGFR. These enzymes participate in angiogenesis and the inhibition of angiogenesis most likely plays an important role in the therapeutic response of solid tumors. It will be difficult to establish the precise mechanism of action of dasatinib in the cancer setting owing to its inhibition of several important protein kinases. No X-ray structural studies of dasatinib bound to either PDGFRα or PDGFRβ have been reported. However, we were able to generate a satisfactory pose of the drug binding to PDGFRα using the Schrödinger induced-fit docking protocol [95] with 5GRN (PDB: ID) as a template. The best pose shows that the thiazole *N*3 forms a hydrogen bond with the N—H group of the hinge C677 while the adjacent amino group forms a hydrogen bond with the C677 carbonyl group. Moreover, the carboxamide *N*—H forms a hydrogen bond with the —OH group of the T674 gatekeeper residue (Fig. 8B). The drug interacts hydrophobically with residues near the top of the adenine pocket including L599 at the end of the β1-strand, V607 at the beginning of the β2-strand and V624, A625 (CS8) and V626 within the β3-strand. Dasatinib also makes hydrophobic contact with V658 (Sh1) in the back loop, with I672 within the β5-strand, and with Y676 of the hinge. It also interacts hydrophobically with L825 (CS6) of the β7-strand and C835 which precedes DFG-D836 of the activation segment. Moreover, the drug makes van der Waals contact with F678 within the hinge. The 2-hydroxyethylpiperazine group extends into the solvent. The drug binds to the front pocket, the gate area, and the back pocket including the BP-I-A and BP-I-B subpockets. Dasatinib interacts with the DFG-D_{out} conformation of PDGFRα and is classified as a type II inhibitor [84].

Imatinib (4-[(4-methylpiperazin-1-yl)methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl]benzamide) (Fig. 7C) is a BCR-Abl, Kit, and PDGFR antagonist that is FDA

Table 5
Selected properties of PDGFR multikinase inhibitors.

Name, code, trade name®	Selected targets	PubChem CID[a]	Formula	MW (Da)	D/A[b]	Rotatable bonds	FDA-approved indications (year of initial approval)[c]
Axitinib, AG-013736, Inlyta®	PDGFRα/β, VEGFR1/2/3	6450551	$C_{22}H_{18}N_4OS$	386	2/4	5	RCC (2012)
Dasatinib, BMS-354825, Sprycel®	PDGFRα/β, BCR-Abl, Src, Lck, Yes, Fyn, Kit, EphA2	3062316	$C_{22}H_{26}ClN_7O_2S$	488	3/9	7	Ph^+ CML or ALL (2006)
Imatinib, CGP 57148, STI571, Gleevec®	PDGFRα/β, BCR-Abl, Kit	5291	$C_{29}H_{31}N_7O$	494	2/7	7	Ph^+ CML or ALL, aggressive systemic mastocytosis, CEL, DFSP, HES, GIST, MDS/MPD (2001)
Lenvatinib, AK175809, E7080, Lenvima®	PDGFRα/β, VEGFRs, FGFRs, Kit, RET	9823820	$C_{21}H_{19}ClN_4O_4$	427	3/5	6	Differentiated thyroid cancer (2015)
Nilotinib, AMN107, Tasigna®	PDGFRα/β, BCR-Abl, DDR1	644241	$C_{28}H_{22}F_3N_7O$	530	2/9	6	Ph^+ CML (2007)
Nintedanib, BIBF-1120, Vargatef®	PDGFRα/β, FGFR1/2/3, VEGFR1/2/3, Flt3	9809715	$C_{31}H_{33}N_5O_4$	540	2/7	8	Idiopathic pulmonary fibrosis (2014)
Ponatinib, AP24534, Iclusig®	PDGFRα/β, BCR-Abl, BCR-Abl T315I, VEGFR, FGFR, EphR, Src family kinases, Kit, RET, Tie2, Flt3	24826799	$C_{29}H_{27}F_3N_6O$	533	1/8	6	Ph^+ CML or ALL (2012)
Sorafenib, BAY 43–9006, Nexavar®	PDGFRα/β, B/C-Raf, B-Raf (V600E), Kit, Flt3, RET, VEGFR1/2/3	216239	$C_{21}H_{16}ClF_3N_4O_3$	465	3/7	5	Hepatocellular carcinoma, RCC, differentiated thyroid cancer (2005)
Sunitinib, SU-11248, Sutent®	PDGFRα/β, VEGFR1/2/3, Kit, Flt3, CSF-1R, RET	5329102	$C_{22}H_{27}FN_4O_2$	398	3/4	7	RCC, GIST, pNET (2006)

[a] www.ncbi.nlm.nih.gov/pccompound.
[b] No. of hydrogen bond donors/acceptors.
[c] ALL, acute lymphoblastic leukemia; CEL, chronic eosinophilic leukemia; CML, chronic myelogenous leukemia; DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumors; HES, hypereosinophilic syndrome; MDS/MPD, myelodysplastic/myeloproliferative diseases; Ph^+, Philadelphia chromosome positive; pNET, pancreatic neuroendocrine tumor; RCC, renal cell carcinoma.

approved for the treatment of several myeloproliferative neoplasms and two solid tumors (GIST and DFSP) (Table 5). Imatinib is FDA approved for the treatment of more maladies than any other targeted protein kinase antagonist (www.brimr.org/PKI/PKIs.htm) and may be considered as a broad-spectrum antagonist. It was initially developed to target BCR-Abl, but the first published study of this drug demonstrated that it blocked PDGF signaling [101]. This drug was the first FDA-approved targeted protein kinase inhibitor (2001) and its initial success paved the way for the development and approval of an additional 35 small molecule antagonists that interact directly with the protein kinase domain (www.brimr.org/PKI/PKIs.htm) [59]. Imatinib is also known as CGP 57148, STI-571 (Signal Transduction Inhibitor-571), Gleevec (in the United States), and Glivec (elsewhere).

Because no X-ray structures of imatinib bound to PDGFRα/β are available, we used the Schrödinger induced-fit protocol [95] to model the human PDGFRα-imatinib complex as described above. The derived structure shows that the *N*1 of the drug pyridine group forms a hydrogen bond with the hinge C677 N—H group, the amino N—H group forms a hydrogen bond with the —OH group of the T674 gatekeeper residue, the amide carbonyl group interacts with the N—H group of DFG-D836, the amide N—H group interacts with the αC-E644 carboxylate group, and the *N*4 piperazine forms a hydrogen bond with the carbonyl group of V815 that is in front of the αE-helix and immediately precedes the catalytic loop (Fig. 8C). The drug interacts hydrophobically with residues near the ceiling of the adenine pocket including L599 in the β1-strand, V607 in the β2-strand, and A625 (CS8) and V626 in the β3-strand. It also has hydrophobic interactions with M648 in the αC-helix, V658 (Sh1) of the αC-β4 back loop, I672 in the β5-strand, Y676 within the hinge, C814 in the large lobe about 8 Å below the αC-helix and before the catalytic loop, L825 (CS6) within the β7-strand, C835 proximal to the activation segment, and DFG-F837 (RS2). Imatinib binds to the front pocket, gate area, back pocket, and the BP-I-A, BP-I-B, BP-II-out, and BP-V back subpockets. The drug interacts with the DFG-D_{out} conformation of PDGFRα and is classified as a type II inhibitor [84].

Lenvatinib (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide) (Fig. 8D) is a PDGFR multikinase antagonist that is FDA approved for the treatment of differentiated follicular and papillary thyroid cancers (Table 5). This drug was initially developed as an angiogenesis inhibitor with activity against VEGF receptors, Kit, and PDGF receptors [102]. However, its activity against differentiated thyroid cancers may be related to its inhibition of RET [80]. We used the Schrödinger induced-fit protocol [95] to dock the drug into human PDGFRα as described above. The pose shows that the *N*1 of the quinoline moiety forms a hydrogen bond with the N—H group of the hinge C677, each of the two urea N—H groups forms a hydrogen bond with αC-E644, the carbonyl oxygen of the urea group forms a hydrogen bond with the N—H group of DFG-D836, and the terminal amide group forms a hydrogen bond with the L599 carbonyl group near the top of the adenine binding pocket. Lenvatinib interacts hydrophobically with several residues of PDGFRα; these residues include V607 near the beginning of the β2-strand, the β3-strand A625 (CS8), M648 within the αC-helix, and Y676 of the hinge. The drug makes similar contact with L825 (CS6) in the β7-strand, C834 before the activation segment, and DFG-F837 (RS2). Lenvatinib binds to the front pocket, gate area, and BP-IB and BP-II-out back subpockets as illustrated in Fig. 9. The 7-methoxy group extends into the solvent. The drug interacts with the DFG-D_{out} conformation of PDGFRα and is classified as a type II inhibitor [84].

Nilotinib (4-methyl-*N*-[3-(4-methylimidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]benzamide) (Fig. 7E) is a PDGFR, BCR-Abl, and DDR1/CD167a antagonist that is FDA approved for the treatment of imatinib-resistant Philadelphia chromosome positive chronic myelogenous leukemia. This drug is a congener of imatinib

that binds more tightly to BCR-Abl than imatinib [103]. Liu et al. demonstrated that nilotinib also inhibited the activation of PDGFR [104]. We used the Schrödinger induced-fit protocol [95] to dock the drug into human PDGFRα as described above. The pose shows that the pyridine *N*1 forms a hydrogen bond with the hinge C677 N—H group, the amino group N—H forms a hydrogen bond with the —OH group of the T674 gatekeeper, the amide N—H group forms a hydrogen bond with αC-E644, and the carbonyl group of the amide group forms a hydrogen bond with DFG-D836 (Fig. 8E). Nilotinib interacts hydrophobically with several residues of PDGFRα including L599 at the end of the β1-strand, V607 near the beginning of the β2-strand, β3-strand A625 (CS8) and V626, αC-I647, I657 and V658 (Sh2) in the αC-β4 back loop, I672 in the β5-strand, Y676 within the hinge, and H816 (RS1). The drug also makes hydrophobic contact with L825 (CS6) on the floor of the adenine pocket, I834 and C835 between the β8-strand and activation segment, and DFG-F837 (RS2) of the activation segment. The hydrophobic interactions involving nilotinib are quite extensive; it binds to the front pocket, gate area, and back pockets and the BP-I-A, BP-I-B, BP-II-out, BP-III, and BP-V subpockets. The drug interacts with the DFG-D_{out} conformation of PDGFRα and is classified as a type II inhibitor [84].

Nintedanib (methyl (3Z)-3-[[4-[methyl-[2-(4-methylpiperazin-1-yl)acetyl]amino]anilino]-phenylmethylidene]-2-oxo-1H-indole-6-carboxylate) (Fig. 7F) is a PDGFRα/β, FGFR1/2/3, and VEGFR1/2/3 antagonist that is FDA approved for the treatment of idiopathic pulmonary fibrosis and for the second-line treatment of NSCLC in combination with docetaxel [105,106]. In addition to these protein kinases, Hilberg et al. reported that nintedanib is a potent inhibitor (IC_{50} values less than 100 nM) of nearly two dozen other protein kinases including Abl, BTK, Kit, RET and Yes [106]. Its activity against pulmonary fibrosis may be related to its blockade of both the PDGF receptors and to the FGF receptors, which are signaling pathways related to fibrosis. We used the Schrödinger induced-fit docking protocol [95] to generate a structure of the drug bound to PDGFRα as described above. The resulting pose shows that the indole *N*1 forms a hydrogen bond with the carbonyl group of the first hinge residue (E675), the indole 2-oxo group forms a hydrogen bond with the third hinge residue (C677) N—H group, and the indole 6-carbonyl group hydrogen bonds with the β3-K627 (Fig. 8F). Moreover, the N—H group emanating from the piperazine, which occurs in the solvent, forms an electrostatic bond with β2-E609 on the exterior portion of the kinase domain. Nintedanib interacts hydrophobically with numerous PDGFRα residues near the top of the adenine pocket including L599 at the end of the β1-strand, V607 within the β2-strand, and β3-A625 (CS8). The drug also makes hydrophobic contact with αC-M648, V658 (Sh1) within the back loop, Y676 of the hinge, the β7-strand L825 (CS6) on the floor of the adenine pocket, and DFG-F837 (RS2). Nintedanib binds to the front pocket, the gate area, and the BP-I-B subpocket. The drug interacts with the DFG-D_{out} conformation of PDGFRα and is classified as a type II inhibitor [84].

Ponatinib (3-(2-imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methyl-*N*-[4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl]benzamide) (Fig. 7G) is a PDGF receptor and BCR-Abl multikinase antagonist that is approved for the treatment of Philadelphia chromosome positive acute lymphoblastic and chronic myelogenous leukemias. The drug is a second generation BCR-Abl inhibitor that was developed to inhibit the Abl T315I gatekeeper mutant [107], but the drug inhibits several other protein kinases (Table 5). We used the Schrödinger induced-fit docking protocol [95] to model the interaction of this drug with PDGFRα as described above. The resulting pose indicates that the drug makes five hydrogen bonds with PDGFRα. These include the *N*1 of the imidazopyridazine ring with the carbonyl group of C677 (the third hinge residue), the benzamide carbonyl forms a hydrogen bond with the N—H group of DFG-D836, the N—H of the drug amino group forms a hydrogen bond with αC-E644, and the piperazine N—H group forms hydrogen bonds with the carbonyl groups of V815 and H816 (RS1) (Fig. 8G). Ponatinib interacts hydrophobically with residues near the ceiling of the adenine pocket including L599 at the end of the β1-strand, V607 near the beginning of the β2-strand, and the β3 A625 (CS8) and V626. It makes similar contacts with L651 at the end of the αC-helix, I657 and V658 (Sh1) in the back loop, I672 in the β5-strand, and Y676 within the hinge. Ponatinib also makes hydrophobic contact with L809 in the αE-helix of the large lobe and beneath the back loop, C814 within the large lobe and beneath the αC-helix, L825 (CS6) in the β7-strand, and C835 prior to the activation segment. The drug also makes van der Waals contact with the gatekeeper T674. The drug binds to the front pocket, gate area, and back pockets including the BP-I-A, BP-IB, BP-II-out, BP-III, and BP-IV subpockets (Fig. 9). This structural modeling indicates that ponatinib binds to a DFG-D_{out} conformation of PDGFRα and is thereby classified as a type II inhibitor [84].

Sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methylpyridine-2-carboxamide) (Fig. 7H) is a PDGF receptor multikinase antagonist that is FDA approved for the treatment of hepatocellular carcinomas and differentiated follicular and papillary thyroid carcinomas. This antagonist was initially developed as a Raf inhibitor, but it is a potent antagonist of several other protein kinases (Table 5) [108,109]. We used the Schrödinger induced-fit docking protocol [95] to derive a model for the interaction of this drug with PDGFRα as described above. The resulting pose indicates that the drug makes five hydrogen bonds with PDGFRα. The terminal carboxamide N—H forms a hydrogen bond with the carbonyl group of C677 and the *N*1 of the pyrimidine forms a hydrogen bond with the N—H group of C677 within the hinge. The two urea N—H groups form hydrogen bonds with the carboxyl group of αC-E644 and the urea oxygen forms a hydrogen bond with the N—H group of DFG-D836. Moreover, the drug interacts hydrophobically with residues near the ceiling of the adenine pocket including L599 at the end of the β1-strand, V607 near the beginning of the β2-strand, and the β3-strand A625 (CS8). It makes similar contacts with L651 of the αC-helix, I657 and V658 (Sh1) in the αC-β4 back loop, and Y676 within the hinge. Sorafenib also makes hydrophobic contact with L809 and C814 in the large lobe beneath the αC-helix and before the catalytic loop, H816 (RS1), L825 (CS6) on the floor of the adenine pocket along with I834 and C835 preceding the activation segment. Sorafenib binds to the front pocket and to the BP-I-B back subpocket. Like the previous drugs, ponatinib binds to a DFG-D_{out} conformation of PDGFRα and is thereby classified as a type II inhibitor [84].

Sunitinib (*N*-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide) (Fig. 7I) is a PDGF receptor multikinase antagonist that is FDA approved for the treatment of renal cell carcinomas, GIST, and pancreatic neuroendocrine tumors. Sunitinib was developed as an anti-angiogenesis drug and inhibits PDGF and VEGF receptors, Kit, and Flt3 (Table 5) [110,111]. We used the Schrödinger induced-fit docking protocol [95] to derive a model of the PDGFRα-sunitinib complex as described above. The computer-generated pose indicates that the N—H of the sunitinib indole group amide forms a hydrogen bond with the carbonyl group of the first hinge residue (E675) and the drug amide N—H group forms a hydrogen bond with the carbonyl group of the third hinge residue (C677) (Fig. 8I). The drug also interacts hydrophobically with residues near the ceiling of the adenine pocket including L599 at the end of the β1-strand and A625 (CS8) of the β3-strand. It makes similar contacts with V658 (Sh1) in the back loop, Y676 within the hinge, L825 (CS6) within the β7-strand, C835 immediately before the activation segment, and DFG-F837 (RS2) of the

activation segment. The diethylaminoethyl group extends into the solvent. The drug interacts with the front pocket and BP-I-B in the back pocket. Like the previous drugs, ponatinib binds to a DFG-D_{out} conformation of PDGFRα and is thereby classified as a type II inhibitor [84].

To summarize this section, each of the drugs forms a hydrogen bond with the third hinge residue (C677) and axitinib, nintedanib, and sunitinib also form a hydrogen bond with the first hinge residue (E675). Dasatinib, imatinib, and nilotinib form hydrogen bonds with the —OH group of the T674 gatekeeper. Moreover, axitinib, imatinib, lenvatinib, nilotinib, ponatinib, and sorafenib form hydrogen bonds with αC-E644 and DFG-D836; these are classical type II inhibitor interactions with a target enzyme [90]. All of the drugs interact with L599 at the end of the β1-strand, V658 within the αC-β4 back loop, Y676 within the hinge, and L825 (CS6) on the floor of the adenine pocket; nearly all of the drugs make hydrophobic contact with the β3-strand A625 (CS8). Several of the drugs also make hydrophobic contact with V607 near the beginning of the β2-strand, C835 before the activation segment, and DFG-F837 (RS2). Pazopanib and regorafenib are two additional FDA-approved PDGF receptor multikinase antagonists. However, we were unable to obtain satisfactory poses of them bound to the PDGFRα using the Schrödinger glide or induced-fit programs [95,96].

5. Epilogue

The FDA approved imatinib as an adjuvant treatment after surgery for GIST (www.brimr.org/PKI/PKIs.htm). Resistance generally develops in about two years owing to mutations in *KIT* or *PDGFRA* and regorafenib and sunitinib are FDA approved as second-line therapy for these patients. With time, resistance to all three medications can occur and such patients lack standard treatment options. Several alternative multikinase inhibitors including nilotinib, sorafenib, and others are being tested, but no additional drugs have proven to be clinically efficacious. See Ref. [112] for a comprehensive discussion of new targets, new therapies, and on-going clinical trials for the treatment of GIST. For both practical and scientific reasons, it would be helpful to have specific PDGFR antagonists so that the precise role of this receptor family in the pathogenesis of a variety of illnesses could be studied without being concerned about the blockade of other enzymes by multikinase antagonists. Except for DFSP and *PDGFRA*-mutant GIST, the effectiveness of PDGFR antagonists may be related to their role in blocking signaling pathways in the tumor stroma.

The difference between more active and less active conformations is a significant characteristic when considering the regulation of protein kinase signaling. DFG-$D_{in/out}$ (active/inactive), $\alpha C_{in/out}$ (active/inactive), and the structure of the activation segment as open/closed (active/inactive) represent major conformational states. There are many subtleties in describing more active and less active conformations within this context. Sometimes the distinction of various DFG-$D_{in/out}$ conformations may be difficult to classify by inspection, but we find that the structure of the R-spine and the location of RS2 (DFG-F) removes most of these ambiguities. A linear R-spine characterizes the more active state while a nonlinear or broken R-spine depicts a less active state. Measuring D1 (the distance between the αC atoms of the HRDxxxxN asparagine and the αC atoms of DFG-F) and D2 (the distance between the αC atoms of αC-E and DFG-F) as described by Vijayan et al. removes most of the uncertainty in the DFG-$D_{in/out}$ classification [62].

Although the pose of each drug with its protein kinase target is unique, it is helpful to classify these interactions and apply them to the drug discovery process. We have classified these drugs into seven possible types (I–VI and I½) based upon the structures of the drug-protein kinase complexes [84]. Type I drugs bind to the active conformation of the protein kinase with (i) DFG-D_{in}, (ii) αC_{in}, (iii) an open activation segment, and (iv) a linear R-spine. The type I½ drugs bind to an inactive enzyme conformation with DFG-D_{in}; the activation segment may be closed, the R-spine may be nonlinear, or the αC-helix may be out. We subdivided this class into type I½A drugs that extend into the back hydrophobic pocket while the type I½B drugs do not extend past the gatekeeper [84]. Preliminary data suggest that the type A antagonists have an extended residence time while the type B antagonists do not.

The type II drugs bind to their target enzyme with DFG-D_{out}, which corresponds to a less active conformation [88]. The R-spine RS2 residue is displaced from RS1 and RS3 and the R-spine is broken. The type IIA drugs are localized in the front cleft, the gate area, and the back pocket while the type IIB drugs are localized within the front cleft and gate area and do not extend past the gate area [84]. Owing to the multiplicity of less active protein kinase conformations when compared with the canonical more active conformation, it was hypothesized that type II drugs would be less promiscuous than type I drugs which bind to the typical active conformation. The analysis of Vijayan et al. support this hypothesis [62] while that of Zhao et al. does not [113]. Although type II inhibitors may be more selective, most – if not all – inhibit more than one target protein kinase and the differences are a matter of degree only. The type III inhibitors are allosteric in nature and bind adjacent to the adenine binding pocket [91]. Because there is more variability in this region than in the ATP binding pocket, type III inhibitors may be more selective than type I, I½, or II inhibitors. Of the FDA-approved medicines that have been classified based upon the nature of their binding to their protein kinase target enzymes, the drugs are nearly equally distributed among the type I, I½, and II categories [80,84]. Moreover, there are two type III allosteric inhibitors (cobimetanib and trametinib) and two type VI irreversible inhibitors (afatinib and ibrutinib) that are FDA-approved protein kinase antagonists.

That a given drug can bind to multiple conformations of its protein kinase targets increases the complexity of inhibitor taxonomy [84]. For example, bosutinib is a type I antagonist of the Src protein-tyrosine kinase and a type IIB antagonist of the Abl protein-tyrosine kinase. Crizotinib is a type I inhibitor of the ALK receptor protein-tyrosine kinase and a type I½B inhibitor of c-Met (hepatocyte growth factor receptor protein-tyrosine kinase). Moreover, sunitinib is a type I½B inhibitor of cyclin-dependent protein kinase 2 (a protein-serine/threonine kinase) or CDK2 and a type IIB inhibitor of the stem cell factor receptor protein-tyrosine kinase (Kit) [114,115]. Adding to the complexity, erlotinib is a type I and I½B inhibitor of EGFR/ErbB1 (epidermal growth factor receptor protein-tyrosine kinase). These findings indicate that protein kinase antagonists lack conformational selectivity. Furthermore, these results show that drugs occupying the adenine pocket and gate area while excluding the back cleft may be interchangeable as type I, I½B, and IIB inhibitors. By inference, antagonists that extend into the back cleft may function as type I½A and IIA inhibitors, but not type I inhibitors.

The search for therapeutic protein kinase antagonists began earnestly in 2001 when the US FDA approved imatinib for the treatment of Philadelphia chromosome positive chronic myelogenous leukemia. The original Novartis tradename for imatinib was Glivec. The FDA determined that this might be confused in oral communication with Glyset (miglitol), an oral diabetes drug. The United States tradename was then changed to Gleevec [116]. Because both Glivec and Gleevec are pronounced glee veck, it is difficult to understand how the change in spelling helped to avoid confusion in oral communication unless one thought that Glivec was pronounced gly veck. The FDA has subsequently approved an additional 35 orally effective small molecule antagonists (a total of 36) that directly target a protein kinase domain for the treatment of an assortment of

neoplastic, fibrotic, and inflammatory disorders (www.brimr.org/PKI/PKIs.htm).

Of the 518 human protein kinases, only about three dozen of them have been targeted for the treatment of assorted illnesses. Nearly all of the current therapeutic indications are for neoplastic disorders including an assortment of solid tumors as well as leukemias and lymphomas. The approvals of tofacitinib (a Janus protein-tyrosine kinase family antagonist) for the treatment of rheumatoid arthritis in 2012 and nintedanib (a FGFR, PDGFR, and VEGFR antagonist) for the treatment of idiopathic pulmonary fibrosis in 2014 represent an expanded therapeutic range. Owing to their role in nearly all aspects of intercellular and intracellular signaling, a much greater range of maladies should be amenable to treatments with protein kinase antagonists [117]. Accordingly, we can expect future advances in clinical effectiveness and the subsequent approval of additional drugs targeting (i) more protein kinases and (ii) other illnesses such as atherosclerosis, hypertension, and Parkinson disease. The fundamental research that lead to the discovery of PDGF was based upon its role in promoting the growth of monkey arterial smooth muscle cells in culture in an effort to determine why these cells accumulate in atherosclerosis [1].

Conflict of interest

The author is unaware of any affiliations, memberships, or financial holdings that might be perceived as affecting the objectivity of this review.

Acknowledgments

The colored figures in this paper were evaluated to ensure that their perception was accurately conveyed to colorblind readers [118]. The author thanks Laura M. Roskoski for providing editorial and bibliographic assistance. We also thank Josie Rudnicki and Jasper Martinsek for their help in preparing the figures and Pasha Brezina and W.S. Sheppard for their help in structural analyses.

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