Pharmacol. Ther. Vol. 82, Nos. 2–3, pp. 231–239, 1999

ISSN 0163-7258/99 $–see front matter
PII S0163-7258(98)00066-7

Associate Editor: D. Shugar

Protein Tyrosine Kinase Inhibitors as Novel Therapeutic Agents

Alexander Levitzki

DEPARTMENT OF BIOLOGICAL CHEMISTRY, THE ALEXANDER SILBERMAN INSTITUTE OF LIFE SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, JERUSALEM 91904, ISRAEL

ABSTRACT. Protein tyrosine kinases (PTKs) play a key role in normal cell and tissue development. Enhanced PTK activity is intimately correlated with proliferative diseases, such as cancers, leukemias, psoriasis, and restenosis. This realization prompted us to systematically synthesize tyrosine phosphorylation inhibitors (tyrphostins) as potential drugs. Over the years, we have demonstrated the ability to synthesize selective tyrphostins aimed at different receptor, as well as at nonreceptor, tyrosine kinases. Some of these tyrphostins have shown efficacy *in vivo* as antileukemic agents and antirestenosis agents. AG 490, a Jak-2 inhibitor, is potent against recurrent pre-B acute lymphoblastic leukemia. AG 1295, a selective platelet-derived growth factor receptor kinase inhibitor, inhibits 50% of balloon injury-induced stenosis in the phemoral arteries of pigs. AG 1517 (SU 5271), a potent epiderminal growth factor receptor kinase inhibitor, is currently in clinical trials for psoriasis. Similarly, SU 5416, a potent kinase inhibitor of the vascular endothelial growth factor receptor/kinase domain receptor/Flk-1, is currently in clinical trials as an anticancer agent by virtue of its strong anti-angiogenic activity. These findings demonstrate that the identification of PTKs that play a key role in a defined disease state can lead to a selective drug. Tyrphostins also show efficacy *in vivo* in inflammatory diseases such as sepsis, cirrhosis, and experimental autoimmune encephalitis. PHARMACOL. THER. 82(2–3):231–239, 1999.

KEY WORDS. Protein tyrosine kinases, tyrphostin inhibitors, proliferative diseases, drug development, clinical trials.

CONTENTS

ABBREVIATIONS. CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor type 1 receptor; IL, interleukin; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PTK, protein tyrosine kinase; TNF, tumor necrosis factor; VEGFR, vascular endothelial growth factor receptor.

1. INTRODUCTION

The malfunctioning of protein tyrosine kinases (PTKs) is the hallmark of numerous diseases. By malfunctioning, one usually refers to enhanced activity of the PTK. Over 80% of the oncogenes and proto-oncogenes involved in human cancers code for PTKs. The enhanced activities of PTKs are also implicated in many nonmalignant diseases, such as psoriasis, papilloma, restenosis, and pulmonary fibrosis. PTKs have also been implicated in inflammatory conditions. These realizations have resulted in the surge of publications describing attempts to target PTKs for drug development (for reviews, see Levitzki and Gazit, 1995; Levitzki, 1992, 1996a,b, 1997). Understanding the molecular pathology of many diseases has progressed at an accelerating pace, and currently, one can identify the molecular aberrations that are the hallmarks of an increasing number of pathological states. PTKs occupy a prominent position among the molecular elements gone awry. Table 1 summarizes the PTK signaling molecules whose altered activities have been shown to be directly associated with a human disease.

2. UNIVERSAL TARGETS AND SELECTIVE TARGETS

In most proliferative diseases, more than one signaling pathway is involved. This is especially true in cancers, where many genetic alterations have taken place on the pathway of

not inhibit Ser/Thr kinases was reported in the late 1980s (Yaish *et al.*, 1988). Within less than a decade, highly potent and selective tyrosine kinase inhibitors were produced by a number of groups, mostly by semi-rational drug design (for reviews, see Levitzki, 1992, 1996b, 1997; Groundwater *et al.*, 1996). Detailed kinetic analysis of the mode of EGFR kinase action shows that ATP and the substrate bind independently to the kinase domain (Posner *et al.*, 1992), which simplifies the kinetic analysis of the mode of inhibition of PTKs by their inhibitors (Posner *et al.*, 1994). One of the most surprising findings on the selectivity of inhibitors discovered so far is that ATP competitive inhibitors can be so selective. For example, quinoxalines are highly selective inhibitors of PDGFR kinase (Kovalenko *et al.*, 1994; Gazit *et al.*, 1996a), whereas the not so distant quinozalines are highly selective for the EGFR kinase (Osherov and Levitzki, 1994; Ward *et al.*, 1994; Levitzki and Gazit, 1995; Gazit *et al.*, 1996b). A more detailed analysis of the mode of tyrosine kinase inhibition reveals that the affinity of the inhibitor and its mode of binding to the kinase domain depends on whether the kinase is in its activated form or in its basal inactive state (Levitzki and Bohmer, 1998). Three examples illustrate this point.

(1) Activated Abl kinase, such as $p210^{Bcr\text{-}Abl}$ and $p185^{Bcr\text{-}Abl}$, possess different affinities for both substrates and inhibitors (tyrphostins), as compared with their proto-oncogenic form $p140^{c\text{-}Abl}$ (Anafi *et al.*, 1992). The oncoproteins $p210^{Bcr\text{-}Abl}$ and $p185^{Bcr\text{-}Abl}$ are inhibited by the tyrphostin AG 957 with K_i values of 0.75 μM and 1.5 μM, respectively, as compared with a K_i of 10.0 μM for the cellular wild-type $p140^{c\text{-}Abl}$. AG 957 is competitive with the substrate and noncompetitive with ATP. Interestingly, for all three proteins the difference between c-Abl and Bcr-Abl is not in the kinase domain, since the Bcr sequence is fused in-frame with the intact kinase domain. It seems that tethering the Bcr sequence upstream to the c-Abl alters the conformation of the kinase domain such that it binds more tightly to the inhibitor.

(2) An identical pattern of behavior is observed for the EGFR kinase: the receptor truncated at the extracellular domain, EGFRΔ, exhibits one order of magnitude higher affinity for quinozaline AG 1478 as compared with the wild-type receptor (Han *et al.*, 1996). Here, too, the deletion is in the extracellular domain and not in the kinase domain. The structural alterations, however, that are induced by truncation seem to alter the conformation within the catalytic domain of the receptor. In both the Bcr-Abl case and the EGFRΔ case, these differences need to be studied further for a detailed molecular explanation. These differences, however, can be exploited to kill preferentially the cancer cell, which is more sensitive to the agent. Indeed, we can show that Ph^+ cells can be purged selectively with AG 957, with no significant loss of normal blood tissue cells in bone marrow samples from CML patients (Carlos-Stella *et al.*, 1999).

(3) Another interesting example is the PDGFR kinase. Upon activation of the receptor by PDGF and autophosphorylation, the mode of inhibition by the selective inhibitor AG 1296 (or AG 1295) is altered. Whereas the inhibitor is competitive vis-à-vis ATP with the inactive form of the receptor, it binds with higher affinity and becomes mixed-competitive vis-à-vis ATP, subsequent to receptor activation by PDGF (Kovalenko *et al.*, 1997).

The availability of the three-dimensional structure of an increasing number of kinases will allow a better understanding of these phenomena and will make drug design more precise and rational. The three-dimensional structures of two tyrosine kinases complexed with inhibitors are already available: (1) the structure of the fibroblast growth factor receptor with selective and nonselective inhibitors (Mohammadi *et al.*, 1997) and (2) the Src kinase Hck crystallized with the inhibitor complexed with it (Sicheri *et al.*, 1997; Schindler *et al.*, 1999). These structures currently are guiding a number of laboratories, including ours, in attempts to design novel and more selective Src kinase inhibitors. The availability of the insulin receptor kinase structure in its inactive form (Hubbard *et al.*, 1994), as well as in its active form complexed with APPNHP with a peptide substrate (Hubbard, 1997), should allow an educated search for inhibitors for the IGF-1R kinase, which is highly homologous to the insulin receptor kinase. Some promising lead compounds, such as AG 1024, have already been found (Parrizas *et al.*, 1997). Importantly, some of the inhibitors discriminate between the insulin receptor kinase and the IGF-1R kinase by a factor of up to 8 (Parrizas *et al.*, 1997). It remains a challenge to design inhibitors of IGF-1R kinase that differentiate between the two receptors by a wider margin.

Fig. 1 summarizes the main pharmacophores that have proven to be effective PTK inhibitors with no significant effects on Ser/Thr kinases. Fig. 2 summarizes the specific compounds that have shown biological efficacy on the targets quoted.

4. BIOLOGICAL EFFICACY OF TYRPHOSTINS

A number of families of tyrphostins have excellent efficacy in tissue culture, as well as *in vivo* (Levitzki, 1996b, 1997), e.g., benzenemalononitriles, which comprised the first family of PTK inhibitors (Yaish *et al.*, 1988; Levitzki and Gazit, 1995). Compounds such as AG 490, AG 126, AG 556, and AG 17 have shown efficacy *in vivo* (Meydan *et al.*, 1996; Lopez-Talavera *et al.*, 1997; Sevransky *et al.*, 1997; Vanichkin *et al.*, 1996; Novogrodsky *et al.*, 1994; Golomb *et al.*, 1996). Anilidophthalimides, which inhibit EGFR (Buchdunger *et al.*, 1995a), also show efficacy *in vivo*. Pyrido pyrimidines such as CGP 53716, which inhibit PDGFR (Buchdunger *et al.*, 1995b), have also shown efficacy *in vivo*. A similar compound, CGP 57148B, from the pyrazolo pyrimidines, blocks Bcr-Abl and shows efficacy on intact cells harboring Bcr-Abl (Druker *et al.*, 1996). AG 957, a tyrphos-

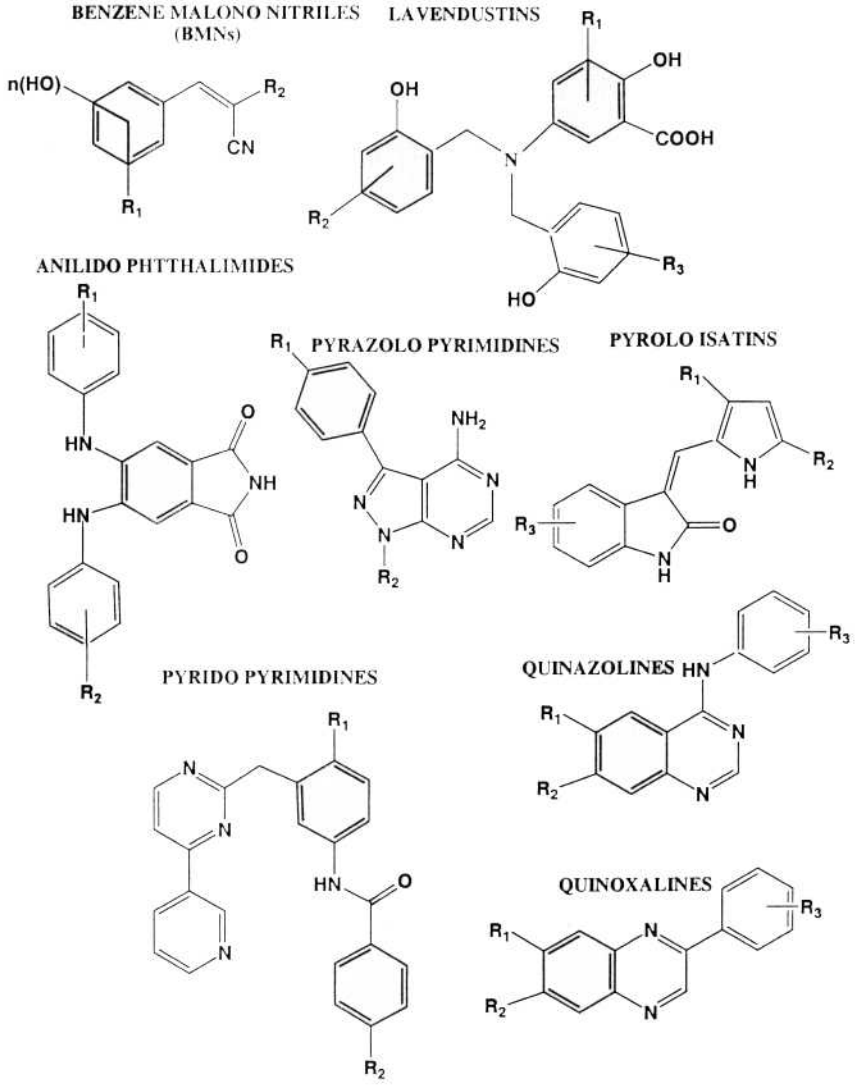

FIGURE 1. Lead pharmacophores for PTK inhibitors.

tin derived from Lavendustin A, is very selective against Bcr-Abl kinase (Anafi *et al.*, 1992; Kaur *et al.*, 1994) and has been shown to purge selectively Ph^+ cells from the blood obtained from CML patients at the chronic phase of the disease. Quinoxalines have shown efficacy against PDGFR kinase (Kovalenko *et al.*, 1994; Gazit *et al.*, 1996a). The quinoxaline AG 1295 has also shown efficacy *in vivo* by inhibiting balloon injury-induced stenosis in the pig (Banai *et al.*, submitted). AG 1295 was also shown to be effective in the rat model (Fishbein *et al.*, submitted). Quinozalines show good efficacy against the EGFR (Ward *et al.*, 1994; Osherov and Levitzki, 1994; Fry *et al.*, 1994; Gazit *et al.*, 1996b; Bridges *et al.*, 1996). One of them, AG 1517 (SU 5271) (Gazit *et al.*, 1996b; Powell *et al.*, submitted), is currently in clinical trials for psoriasis under the name SU 5271. This compound is very effective in blocking the growth of psoriatic keratinocytes (Powell *et al.*, submitted). A related pharmacophore shown to be effective against the EGFR kinase is derived from 4-(phenylamino)-pyrrolopyrimidine (Traxler *et al.*, 1996), a compound that was rationally designed on the basis of successful inhibitors from the dianilinphthalimide family. Yet another successful family of EGFR kinase inhibitors was obtained by the fusion of the quinozaline moiety with a third ring (Sun *et al.*, 1996; see Fig. 2).

5. TYRPHOSTINS ACT IN SYNERGY WITH CYTOTOXIC AGENTS

Enhanced activity of PTKs confers refractoriness of cancer cells towards cytotoxic agents, most probably due to enhancement of anti-apoptotic pathways mediated by Bcl-X_L/Bcl-2 and c-Act/PKB elements. The enhancement of anti-apoptotic pathways seems to be the predominant factor in the emergence of drug resistance and to killing by radiation. In two instances, it was possible to show that inhibiting a PTK by a selective tyrphostin resensitizes the cell to cytotoxic drugs. The HER-2-selective tyrphostin AG 825 was found to synergize with cisplatin, etoposide, and doxorubicin, all DNA-damaging agents, to kill HER-2 overexpressing cells (Tsai *et al.*, 1996). A series of non-small lung cancer cell

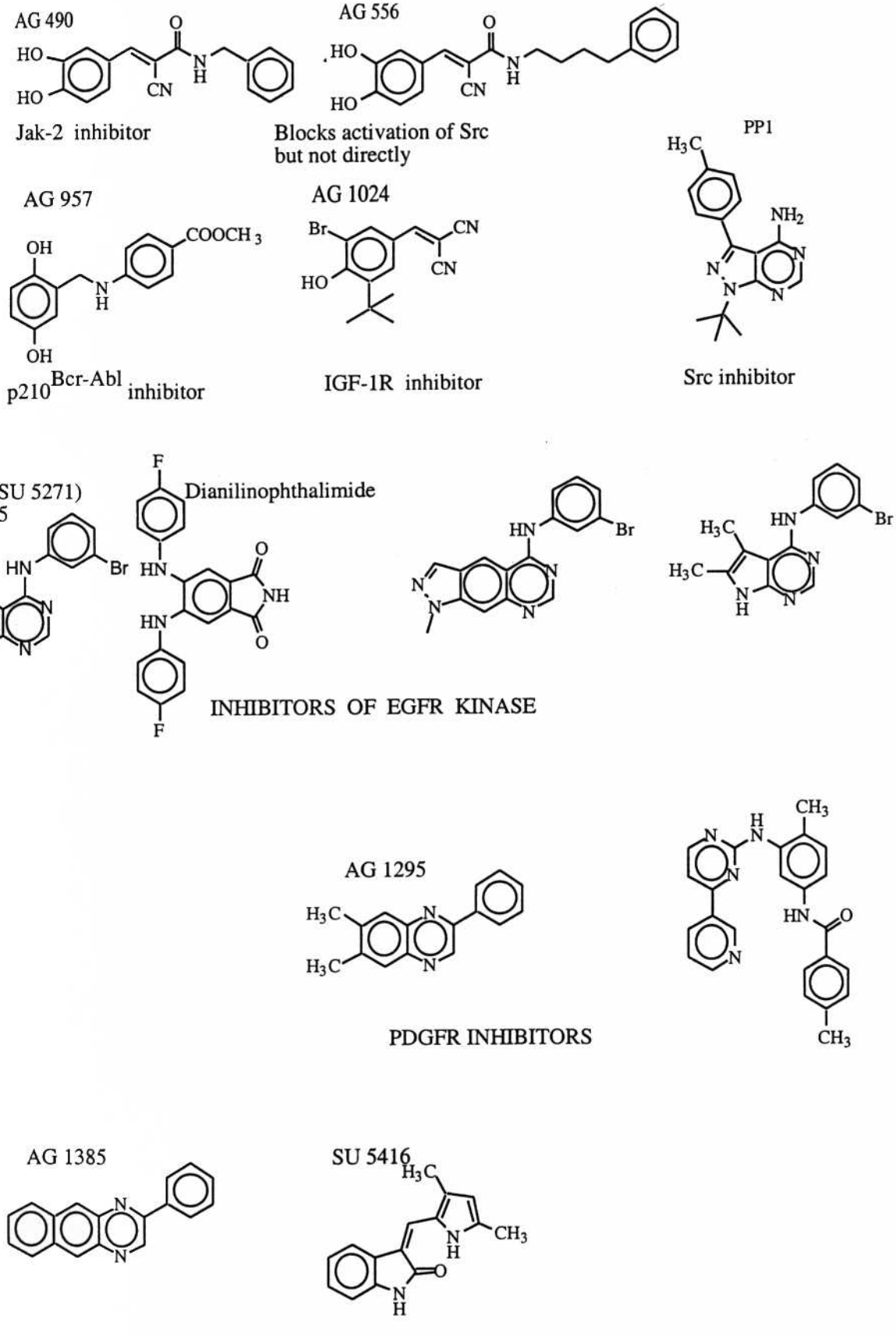

FIGURE 2. Tyrphostins with biological activities.

lines with different levels of HER-2/neu overexpression obtained from lung tumors were examined for the extent of synergy that was found to depend on the level of HER-2/neu expression. It seems that the higher the overexpression of HER-2, the higher the anti-apoptotic shield provided by the receptor tyrosine kinase. At the same time, however, it seems that the sensitivity of these cells to stress signaling is also enhanced upon higher expression of HER-2/neu, but their sensitivity to stress signals seems to be masked by the enhanced anti-apoptotic pathways (M. Benhar *et al.*, submitted). Thus, when the cells are stripped of the robust anti-apoptotic shield by selective tyrphostin blocking of the HER-2/neu signaling, their enhanced vulnerability to stress is exposed. A similar phenomenon was observed for human glioma cells, which overexpress the truncated form of the EGFR—EGFRΔ. The EGFR kinase-selective tyrphostins AG 1478 and AG 1517 synergize with cisplatin to induce apoptosis in the EGFRΔ-overexpressing glioma cells. It was found that AG 1478 reduces the level of Bcl-X_L expression and increases the activity of caspase 3. The combination of a tyrphostin with cisplatin was found to be lethal to the tumor cells, leading to apoptosis (Nagane *et al.*, 1998). EGFRΔ transmits strong anti-apoptotic signals, which, when inhibited, sensitize the cells to the stress signaling triggered by cisplatin. Recently, the combination of AG 1478 and cisplatin was found to cause tumor inhibition when injected into nude mice with xenografts of glioma. Either AG 1478 or cisplatinum alone had no effect (M. Nagane *et al.*, unpublished). This success hopefully will be translated into clinical application of these tyrphostins as sensitizers of these tumors to cytotoxic drugs, to which they are normally oblivious. This type of combinatorial approach, which combines a blocker of anti-apoptotic signaling with a stimulator of stress signaling, could prove to be a more general approach.

6. SUCCESS OF TYRPHOSTINS *IN VIVO*

Some tyrphostins already have shown efficacy *in vivo* in preclinical models.

6.1. *Platelet-Derived Growth Factor Receptor Kinase-Directed Protein Tyrosine Kinase Inhibitors*

Tyrphostin AG 1295 and its close analog AG 1296, both quinoxalines (Fig. 1), have been shown to block selectively PDGFR kinase with insignificant inhibitory effects on EGFR, Src, Flk-1, HER-2/neu, and IGF-1R. These tyrphostins reverse the transformed phenotype of *sis*-transformed NIH 3T3 cells (Kovalenko *et al.*, 1994) and slow C6 glioma-induced tumors in nude mice (L. Shawver and A. Levitzki, unpublished). In recent experiments on pigs, AG 1295 was shown to block balloon injury-induced stenosis in the femoral artery (Banai *et al.*, 1998). The extent of inhibition is ~50–60% compared with balloon-injured femoral artery treated with the vehicle alone. Similar results were obtained for rats. AG 17, a nonselective PTK inhibitor, was successful in blocking balloon injury-induced stenosis in the rat carotid artery model (Golomb *et al.*, 1996), but induced damage in pigs (S. Banai and A. Levitzki, unpublished). The pig model, which is much more faithful and predictive for the human situation, encourages one to promote AG 1295 and its more potent analogs (A. Gazit, S. Banai, and A. Levitzki, in preparation) to undergo testing in the clinical set-up. The ability of very selective PDGFR kinase inhibitors to block balloon injury-induced stenosis supports what has been known as "the PDGF hypothesis" of Ross (Ross and Glomset, 1976; Rewcastle *et al.*, 1986), which implicates this growth factor and its receptor in the process of atherosclerosis and its accelerated form that takes place in restenosis. The finding that PDGFR kinase-directed tyrphostins block ~60% stenosis strongly suggests that other signaling pathways should be targeted if one would like to block the other proliferative signals, including those elicited by fibroblast growth factor and transforming growth factor-α. Since accelerated atherosclerosis in the transplanted heart is the major cause of death of patients who undergo heart transplantation, AG 1295 and its analogs currently are being tested in an animal heart transplant model.

6.2. *Jak-2-Directed Tyrphostins*

Among the many tyrphostins tested, members of the AG 490 family have been shown to be potent inhibitors of Jak-2 (Meydan *et al.*, 1996) and, to a lesser extent, Jak-3 (Sharfe *et al.*, 1995). The high potency of AG 490 to block Jak-2 is likely to be the origin of the ability of these agents to block recurrent pre-B acute lymphoblastic leukemia when engrafted in SCID mice (Meydan *et al.*, 1996). AG 490 induces apoptotic death in pre-B cells from patients suffering from the disease. In these pre-B cells, one finds that Jak-2 is persistently activated, whereas in normal B cells, it is not. It is still unknown whether the persistent activation of Jak-2 is due to an activating mutation or to a robust autocrine stimulation. The efficacy of AG 490 *in vivo* is gratifyingly accompanied by its complete absence of toxicity to normal blood tissue. This agent currently is undergoing evaluation for clinical trials. More recently, AG 490 was also shown to block the survival signaling induced by interleukin (IL)-6 on multiple myeloma. We recently have found that IL-6 induces Stat3 phosphorylation, which, in turn, activates Bcl-X_L expression through binding to its promoter, thus explaining why IL-6 has an anti-apoptotic activity. AG 490 was found to synergize with CH-11, a Fos receptor agonistic antibody, to kill the IL-6-dependent multiple myeloma leukemic cells (Catlett-Falcone *et al.*, 1999).

6.3. *Epidermal Growth Factor Receptor Kinase Inhibitors*

EGFR kinase blockers from the early tyrphostin family had already been shown to be efficacious in 1991, being the first PTK blockers to show good efficacy *in vivo* (Yoneda *et al.*, 1991). Due to their unfavorable pharmacokinetic properties, however, they did not make further progress in preclin-

ical animal models and were abandoned as possible candidates for the clinical setting. These experiments, however, were a milestone in the sense that they provided a proof of principle by showing for the first time that the activity of a tyrphostin identified in a cell-free system culminated in success *in vivo*. A few heterocyclic EGFR kinase inhibitors, such as diamidinephenylindole (Buchdunger *et al.*, 1995a) and CGP 59326A, have shown good efficacy *in vivo* against tumors that overexpress EGFR. One quinazoline, AG 1517 (SU 5271) (Powell *et al.*, submitted), currently is being tested as an antipsoriatic agent. Since papilloma (HPV16)-infected keratinocytes are driven by the EGFR kinase system (Ben-Bassat *et al.*, 1997), success in the psoriasis clinical trials may lead to the testing of EGFR-directed PTK inhibitors as anti-papilloma agents.

6.4. *Vascular Endothelial-Growth Factor Receptor Kinase Inhibitors*

Because of the key role vascular endothelial growth factor receptor (VEGFR)/kinase domain receptor/Flk-1 plays in angiogenesis, inhibitors of the receptor that show efficacy *in vivo* have been synthesized (Strawn *et al.*, 1996). A number of pharmaceutical companies, including Novartis (Basel, Switzerland) and Sugen (Redwood City, CA, USA), have already announced the beginning of clinical trials, using VEGFR kinase inhibitors. Angiogenesis inhibitors are likely to become universal anticancer agents (Levitzki, 1996b, 1997).

7. TYRPHOSTINS AS ANTI-INFLAMMATORY AGENTS

A number of benzenemalononitrile tyrphostins, such as AG 126 (Novogrodsky *et al.*, 1994) and AG 556 (Sevransky *et al.*, 1997; Vanichkin *et al.*, 1996), have shown excellent efficacy as anti-inflammatory agents, inhibiting sepsis in mice and dogs (Vanichkin *et al.*, 1996; Sevransky *et al.*, 1997). More recently, we found that AG 556 is very effective in alleviating the symptoms of experimental autoimmune encephalitis, even on late administration (Brenner *et al.*, 1998). AG 126 was found to alleviate cirrhosis-like symptoms in rats (Lopez-Talavera *et al.*, 1997). The search for such compounds within the tyrphostin family was based on scattered reports in the literature that the activation of PTKs is on the pathway of lipopolysaccharide-induced mortality in mice. Similarly, scattered reports suggest that the action of tumor necrosis factor (TNF)-α involves PTK activation (Kelly *et al.*, 1998) at some point on the overall signaling pathways triggered by the cytokine. Since no specific molecular target has been defined, screening for active tyrphostins was based on the potency of the compounds tested to block lipopolysaccharide-induced TNF-α production (Novogrodsky *et al.*, 1994; Brenner *et al.*, 1998) and TNF-α action (Novogrodsky *et al.*, 1994). It remains to be seen whether the biological activity of this family of tyrphostins is due exclusively to the inhibition of PTKs. More recent evidence from our laboratory suggests that these tyrphostins are also potent lipooxygenase inhibitors (I. Fridriech *et al.*, submitted).

8. CONCLUSIONS AND PERSPECTIVES

In recent years, the network of signal transduction pathways has been largely deciphered. In the process, it has become apparent that aberration in signaling elements is the root of many diseases. Thus, for example, it has become clear very early on that over 80% of all oncogenes and proto-oncogenes code for PTKs. This realization has identified these proteins as potential targets for disease therapy. It is, therefore, no wonder that tyrphostins were the first signal transduction agents to be used in the clinic. In the meantime, the aberrations in many other elements of signal transduction pathways have been identified as the hallmarks of pathological situations, identifying them as novel targets for disease management. Thus, the concept of "signal transduction therapy" (Levitzki, 1994) takes on a broader meaning and can apply to proliferative diseases, such as cancers, psoriasis, and restenosis, as well as inflammatory conditions, such as sepsis and multiple sclerosis. It is highly likely that many more agents that directly modulate signal transduction pathways will be used in the clinic in the near future.

It is very likely that gene therapy targeted to modulate the expression of signaling proteins will also develop in the near future. Some attempts in this direction already have been reported (Indolfi *et al.*, 1995). However, in the absence of good delivery systems, this field is waiting for technological breakthroughs.

References

Anafi, M., Gazit, A., Gilon, C., Ben-Neriah, Y. and Levitzki, A. (1992) Selective interactions of transforming and normal Abl proteins with ATP, tyrosine copolymer substrates and tyrphostins. J. Biol. Chem. 267: 4518–4523.

Bacus S. S., Zenick, C. R., Plowman, G. and Varden, Y. (1994) Expression of the erbB-2 family of growth factor receptors and their ligands in breast cancers. Implication for tumor biology and clinical behavior. Am. J. Clin. Pathol. 102: S13–S24.

Banai, S., Wolf, G., Golomb, A., Pearle, A., Waltenberger, J., Fibstein, I., Schneider, A., Gazit, A., Perez, L. and Huber, R. (1998) PDGF-receptor tyrosine kinase blocker AG 1295 selectively attenuates smooth muscle cell growth in vitro and reduces neointimal formation after balloon angioplasty in swine. Circulation 19: 1960–1969.

Ben-Bassat, H., Vardi, D. V., Gazit, A., Klaud, S. N., Chaouat, M., Hartzstark, Z. and Levitzki, A. (1995) Tyrphostins suppress the growth of psoriatic keratinocytes. Exp. Dermatol. 4: 82–84.

Ben-Bassat, H., Rosenbaum-Mitrani, S., Hartzstark, Z., Shlomai, Z., Kleinberger-Doron, N., Gazit, A., Plowman, G., Levitzki, R., Tsvieli, R. and Levitzki, A. (1997) Blockers of the EGF receptor and of Cdk2 activation induce growth arrest, differentiation, and apoptosis of HPV 16 immortalized human keratinocytes. Cancer Res. 57: 3741–3750.

Brenner, T., Poradosu, E., Soffer, D., Sicsic, C., Gazit, A. and Levitzki, A. (1998) Suppression of experimental autoimmune encephalomyelitis by tyrphostin AG-556. Exp. Neurol. 154: 489–498.

Bridges, A. J., Zhou, H., Cody, D. R., Rewcastle, G. W., McMichael, A., Showalter, H. D. H., Fry, D. W., Kraker, A. J. and Denny, W. A. (1996) Tyrosine kinase inhibitors. 8. An unusually steep structure-activity relationship for analogues of

4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035). A potent inhibitor of the epidermal growth factor receptor. J. Med. Chem. 39: 267–276.

Buchdunger, E., Mett, H., Trinks, U., Regenass, U., Muller, M., Meyer, T., Beilstein, P., Wirz, B., Schneider, P. and Traxler, P. (1995a) 4,5-Bis(4-fluoroanilino)phthalimide: a selective inhibitor of the epidermal growth factor receptor signal transduction pathway with potent in vivo antitumor activity. Clin. Cancer Res. 1: 813–821.

Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Muller, M., Regenass, U. and Lydon, N. (1995b) Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. Proc. Natl. Acad. Sci. USA 92: 2558–2562.

Carlos-Stella, C., Regazzi, E., Sammarelli, G., Colla, S., Garau, D., Gazit, A., Savoldo, B., Cilloni, D., Tabilio, A., Levitzki, A. and Rizzoli, V. (1999) Effects of the tyrosine kinase inhibitor AG957 and an antifas receptor antibody on CD34$^+$ chronic myelogenous leukemia progenitor cells. Blood, in press.

Carroll, M., Tomasson, M., Barker, G., Golub, T. and Gillialand, D. (1996) The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc. Natl. Acad. Sci. USA 10: 14845–14850.

Catlett-Falcone, R., Landowski, T. H., Oshiro, M. M., Turkson, J., Levitzki, A., Savino, R., Ciliberto, G., Moscinski, L., Fernandez-Luna, J. L., Nunez, G., Dalton, W. S. and Jove, R. (1999) Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 10: 105–115.

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J. and Lydon, N. B. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nature Med. 2: 561–566.

Dvir, A., Milner, Y., Chomsky, O., Gilon, C., Gazit, A. and Levitzki, A. (1991) The inhibition of EGF-dependent proliferation of keratinocytes by tyrosine kinase blockers. J. Cell Biol. 113: 857–865.

Fry, D. W., Kraker, A. J., McMichael, A., Ambroso, L. A., Nelson, J. M., Leopold, W. R., Connors, R. W. and Bridges, A. J. (1994) A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. Science 265: 1093–1095.

Gazit, A., App, H., McMahon, G., Chen, J., Levitzki, A. and Bohmer, F. D. (1996a) Tyrphostin. 5. Potent inhibitors of PDGF receptor tyrosine kinase. SAR in quinixalines, quinolines and indole tyrphostins. J. Med. Chem. 39: 2170–2177.

Gazit, A., Chen, J., App, H., McMahon, G., Hirth, P., Chen, I. and Levitzki, A. (1996b) Tyrphostin. 4. Highly potent inhibitors of EGF receptor kinase. Structure activity relationship study of 4-anilido quinazolines. Bioorg. Med. Chem. 8: 1203–1207.

Golomb, G., Fishbein, I., Banai, S., Mishaly, D., Msocovitz, D., Gertz, D., Gazit, A. and Levitzki, A. (1996) Controlled delivery of a tyrphostin inhibits intimal hyperplasia in rat carotid artery injury model. Atherosclerosis 125: 171–182.

Groundwater, P. W., Solomons, K. R. H., Drewe, J. A. and Munawar, M. A. (1996) Protein tyrosine kinase inhibitors. Prog. Med. Chem. 33: 233–329.

Han, Y., Caday, C. G., Nanda, A., Cavenee, W. K. and Su Hunag, H.-J. (1996) Tyrphostin AG 1478 preferentially inhibits human glioma cells expressing truncated rather than wild-type epidermal growth factor receptors. Cancer Res. 56: 3859–3861.

Hubbard, S. R. (1997) Crystal structure of the activated insulin receptor tyrosine kinase, in complex with peptide substrate and ATP analog. EMBO J. 16: 5572–5581.

Hubbard, S. R., Wei, L., Ellis, L. and Hendrickson, W. A. (1994) Crystal structure of the tyrosine kinase domain of the human insulin receptor. Nature 372: 746–754.

Indolfi, C., Avvedimento, E. V., Rapacciuolo, A., Di Lorenzo, E., Esposito, G., Stabile, E., Feliciello, A., Mele, E., Giuliano, P., Condorelli, G. and Chiariello, M. (1995) Inhibition of cellular ras prevents smooth muscle cell proliferation after vascular injury in vivo. Nature Med. 1: 541–545.

Kaur, G., Gazit, A., Levitzki, A., Stowe, E., Cooney, D. A. and Sausville, E. A. (1994) Tyrphostin induced growth inhibition: correlation with effect on p210$^{bcr\text{-}abl}$ autokinase activity in K562 chronic myelogenous leukemia. Anticancer Drugs 5: 213–222.

Kelly, S. A., Goldschmidt-Clermont, P. J., Milliken, E., Arai, T., Smith, E. H. and Bulkley, G. B. (1998) Protein tyrosine phosphorylation mediated TNF-induced endothelial-neutrophil adhesion in vitro. Am. J. Physiol. (Heart Circ. Physiol.) 43: H513–H519.

Kovalenko, M., Gazit, A., Bohmer, A., Rorsman, C., Ronnstrand, L., Heldin, C., Waltenberger, J., Böhmer, F. D. and Levitzki, A. (1994) Selective platelet-derived growth factor receptor kinase blockers reverse sis-transformation. Cancer Res. 54: 6106–6114.

Kovalenko, M., Romstrand, L., Heldin, C.-H., Loubochenko, M., Gazit, A., Levitzki, A. and Bohmer, F. D. (1997) Phosphorylation site-specific inhibition of platelet-derived growth factor b-receptor autophosphorylation by the receptor blocking tyrphostin AG 1296. Biochemistry 36: 6260–6269.

Levitzki, A. (1992) Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. FASEB J. 6: 3275–3282.

Levitzki, A. (1994) Signal transduction therapy. Eur. J. Biochem. 226: 1–13.

Levitzki, A. (1996a) SRC as a target for anti-cancer drugs. Anticancer Drug Des. 11: 175–182.

Levitzki, A. (1996b) Targeting signal transduction for disease therapy. Curr. Opin. Cell. Biol. 8: 239–244.

Levitzki, A. (1997) Targeting signal transduction for disease therapy. Med. Oncol. 14: 83–89.

Levitzki, A. and Bohmer, F. D. (1998) Altered efficacy and selectivity of tyrosine kinase inhibitors of the activated states of protein tyrosine kinases. Anticancer Drug Des. 13: 731–734.

Levitzki, A. and Gazit, A. (1995) Tyrosine kinase inhibition: an approach to drug development. Science 267: 1782–1788.

Lopez-Talavera, J. C., Levitzki, A., Martinez, A., Gazit, A., Esteban, E. and Guardian, J. (1997) Tyrosine kinase inhibition ameliorates the hyperdynamic state and decreases nitric oxide production in cirrhotic rats with portal hypertension and ascites. J. Clin. Invest. 100: 664–670.

Meydan, N., Grunberger, T., Dadi, H., Shahar, M., Arpaia, E., Lapidot, Z., Leader, S., Freedman, M., Cohen, A. and Gazit, A. (1996) Inhibition of recurrent human pre-B acute lymphoblastic leukemia by Jak-2 tyrosine kinase inhibitor. Nature 379: 645–648.

Mohammadi, M., McMahon, G., Sun, L., Tang, C., Hirth, P., Yeh, B. K., Hubbard, S. R. and Schlessinger, J. (1997) Structure of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science 276: 955–960.

Nagane, M., Levitzki, A., Cavenee, W. K. and Su Huang, H.-J. (1998) Drug resistance of human glioblastoma cells conferred

by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases. Proc. Natl. Acad. Sci. USA 95: 5724–5729.

Novogrodsky, A., Vanichkin, M., Patya, A., Gazit, N., Osherov, N. and Levitzki, A. (1994) Prevention of LPS-induced lethal toxicity by tyrosine kinase blockers. Science 264: 1319–1322.

Osherov, N. and Levitzki, A. (1994) Epidermal growth factor dependent activation of Src family kinases. Eur. J. Biochem. 225: 1047–1053.

Parrizas, M., Gazit, A., Levitzki, A., Wertheimer, E. and LeRoith, D. (1997) Specific inhibition of insulin-like growth factor-1 and insulin receptor tyrosine kinase activity and biological functions by tyrphostins. Endocrinology 138: 1427–1433.

Posner, I., Engel, M. and Levitzki, A. (1992) Kinetic model for the epidermal growth factor (EGF) receptor tyrosine kinase and a possible mechanism of its activation by EGF. J. Biol. Chem. 267: 20638–20647.

Posner, I., Engel, M., Gazit, A. and Levitzki, A. (1994) Kinetics of inhibition of the tyrosine kinase activity of the epidermal growth factor receptor and analysis by a new computer program. Mol. Pharmacol. 45: 673–683.

Rewcastle, G. W., Palmer, B. D., Bridges, A. J., Showalter, H. D. and Ross, R. (1986) The pathogenesis of atherosclerosis—an update. N. Engl. J. Med. 314: 488–500.

Ross, R. and Glomset, J. A. (1976) The pathogenesis of atherosclerosis. N. Engl. J. Med. 295: 369–377.

Schindler, T., Sicheri, F., Pico, A., Gazit, A., Levitzki, A. and Kuriyan, J. (1999) Crystal structure of Hck in complex with a Src family-selective tyrosine kinase inhibitor. Mol. Cell., in press.

Sevransky, J. E., Shaked, G., Novogrodsky, A., Levitzki, A., Gazit, A., Hoffman, Z. and Quezado, B. D. (1997) Tyrphostin AG 556 improves survival and reduces multiorgan failure in canine *E. coli* peritonitis. J. Clin. Invest. 99: 1966–1973.

Sharfe, N., Dadi, H. K. and Roifman, C. M. (1995) JAK 3 protein kinase mediates interleukin-7 induced activation of phosphatidyl insositol-3-kinase. Blood 86: 2077–2085.

Sicheri, F., Moarefi, I. and Kuriyan, J. (1997) Crystal structure of the Src family tyrosine kinase Hck. Nature 385: 602–609.

Strawn, L. M., McMahon, G., App, H., Schreck, R., Kuchler, W. R., Longhi, M. P., Hui, T.-H., Tang, C., Levitzki, A., Gazit, A., Chen, I., Keri, G., Orfi, L., Risau, W., Flamme, I., Ullrich, A., Hirth, K. P. and Shawver, L. K. (1996) Flk-1 as a target for tumor growth inhibition. Cancer Res. 56: 3540–3545.

Sun, L., Nelson, J., McMichael, A., Kraker, A. J., Fry, D. W. and Denny, W. A. (1996) Tyrosine kinase inhibitors. 9. Synthesis and evaluation of fused tricyclic quinazoline analogues as ATP site inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor. J. Med. Chem. 39: 918–928.

Traxler, P. M., Furet, P., Mett, H., Buchdunger, E., Meyer, T. and Lydon, N. (1996) 4-(Phenylamino)pyrrolopyrimidines: potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. J. Med. Chem. 39: 2285–2292.

Tsai, C. M., Levitzki, A., Wu, L.-H., Chang, K.-T., Cheng, C.-C., Gazit, A. and Pemg, R.-P. (1996) Enhancement of chemosensitivity by tyrphostin AG 825 in high-p185neu expressing non-small cell lung cancer cells. Cancer Res. 56: 1068–1074.

Vanichkin, A., Palya, M., Gazit, A., Levitzki, A. and Novogrodsky, A. (1996) Late administration of a lipophilic tyrosine kinase inhibitor prevents lipopolysacharide and *Escherichia coli*-induced lethal toxicity. J. Infect. Dis. 173: 927–933.

Ward, W. H. J., Cook, P. N., Slater, A. M., Davies, D. H., Holdgate, G. A. and Gree, L. R. (1994) Epidermal growth factor receptor tyrosine kinase investigation of catalytic mechanism, structure-based searching and discovery of a potent inhibitor. Biochem. Pharmacol. 48: 639–666.

Yaish, P., Gazit, A., Gilon, C. and Levitzki, A. (1988) Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. Science 242: 933–935.

Yoneda, T., Lyall, R. M., Alisina, M. M., Pearsons, P. E., Spada, A. P., Levitzki, A., Zilberstein, A. and Mundy, G. R. (1991) The antiproliferative effects of tyrosine kinase inhibitors tyrphostins on a human squamous cell carcinoma *in vitro* and in nude mice. Cancer Res. 51: 4430–4435.