CHIMIA **2004**, *58*, No. 9

Chimia 58 (2004) 608–612

ISSN 0009–4293

Drug Discovery in Oncology

Carlos García-Echeverría*

Abstract: A deeper understanding of the molecular events leading to tumour formation, invasion, angiogenesis, and metastasis has provided a new mechanistic basis for oncology drug discovery: targeted anticancer therapy. By specifically blocking the molecular pathways implicated in the pathogenesis of cancer, targeted anticancer agents are expected to alter the natural course of the disease and, at the same time, to offer an enhanced therapeutic index over traditional cytotoxic agents. Following this new paradigm, extracellular matrix remodelling enzymes, growth-factor receptors, signal transduction proteins, and regulators of cell-cycle and gene expression have been the subject of intense drug discovery activities. Three representative areas of research in which targeted cancer therapy has advanced with some success have been selected to briefly illustrate the current status and some of the challenges of drug discovery in oncology.

Keywords: Cancer · Drug · Medicinal chemistry · Oncology · Therapy

1. Introduction

Cancer is a general term that covers over 100 different malignancies. These pathogenic conditions are characterized by uncontrolled cellular proliferation and growth, and, under special conditions, tumour cell migration and spread to other organs and tissues. Different factors (*e.g.* genetic predisposition, environmental agents, radiation, age or viruses) can transform normal cells into cancerous ones by altering the normal function of a wide spectrum of regulatory, apoptotic and signal transduction pathways. The complexity of these biochemical processes and networks represents a major challenge in the development of effective and specific cancer therapies, but recent progress in molecular biology has allowed some of the principles underlying tumour cell transformation and growth to be unravelled. This knowledge has impelled a new paradigm in oncology drug discovery: the identification and development of mechanism-based inhibitors of specific biochemical processes that are essential for the malignant phenotype of cancer cells. The rationale behind this approach is relatively simple: specific inhibitors of proteins involved in aberrant signalling mechanisms would interfere with cancer progression, altering the natural course of the disease. Following this paradigm, many different approaches have been attempted and many disappointments have been harvested [1–3]. Due to space constrains, the current status and challenges of three representative areas of research in which targeted cancer therapy has advanced with some success, are briefly reviewed herein.

2. Inhibitors of Tyrosine and Threonine/Serine Kinases

Protein kinases are enzymes that catalyse the phosphorylation of hydroxyl groups on tyrosine, serine or threonine residues. The biological consequences of this seemingly simple enzymatic activity are staggering in tumour cells. Neoplastic transformation, tumour cell growth, survival, angiogenesis, and metastasis are involved, one way or another, in the action of members of this class of enzymes.

Over the years, a set of protein kinases has been selected as therapeutic targets in oncology based on their over-expression/dysfunction in tumour cells or through their association in signal transduction/cell cycle pathways relevant for the malignant phenotype of the cell [4][5]. The critical role of these kinases in the pathophysiology of these certain types of tumours offers a unique opportunity for therapeutic intervention. Thus, specific inhibition of the critical kinase should lead to a modification of the functional response and, in turn, an alteration of the malignant process in question.

Much of the medicinal chemistry effort to inhibit kinases has been directed to interfere with ATP binding. Initially, this approach was considered unlikely to result in useful anticancer drugs due to two main assumptions: i) the ATP-binding site is highly conserved among protein kinases; and ii) the observed Michaelis K_m values of kinases for ATP are often in the 1 to 10 μM range and the compounds would have to compete with intracellular ATP concentrations around 1 to 5 mM. The potent and selective kinase inhibitors available today have confirmed that, at least for some kinases, these initial concerns were not well founded.

Over 30 kinase inhibitors are known at this moment to be undergoing clinical trials for blood neoplasias and solid tumours [4], and two of them, imatinib mesylate (**1**, Fig. 1) [6] and gefitinib (**2**, Fig. 1) [7], have received marketing approval for the treatment of cancers in which the importance of the targeted kinase is well established. In this respect, imatinib mesylate can still be considered the most representative example of a molecular targeted therapy [8]. This phenylamino-pyrimidine, which blocks the kinase activity of v-Abl, c-Kit and PDGFR [9][10], has had a major impact on the treatment of chronic myelogenous leukaemia as well as other tumours (*e.g.* gastro-intestinal stromal tumour) with etiologies based on the activation of its protein targets [11].

Correspondence: Dr. C. Garcia-Echeverria
Novartis Institutes for BioMedical Research
Global Discovery Chemistry-Oncology
WKL-136.4.84
CH–4002, Basel
Tel.: + 41 61 696 10 94
Fax: + 41 61 696 62 46
E-Mail: carlos.garcia-echeverria@pharma.novartis.com

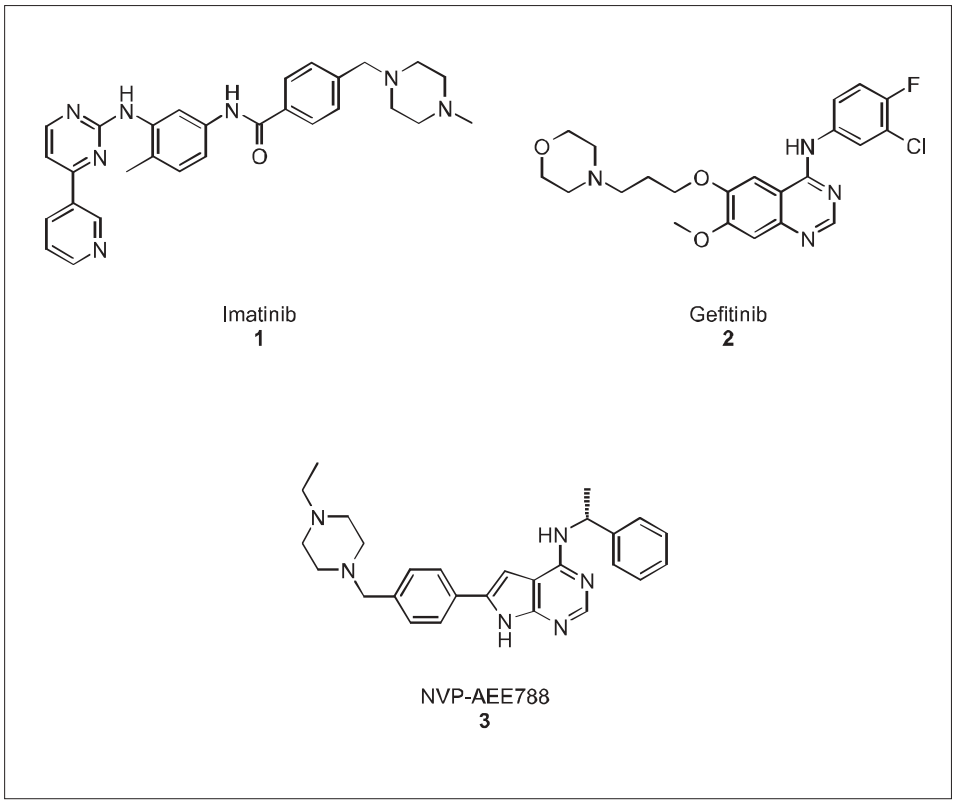

Fig. 1. Representative examples of kinase inhibitors.

The complexity of the kinases being targeted in oncology drug discovery has greatly increased over the past few years, and the major challenges for medicinal chemistry in this area of research will continue to be to identify new chemotypes and to obtain the desired activity and specificity profile, but probably in a different way. If in the past the emphasis was to achieve high selectivity, new directions point to the potential benefit of combining activity against different targets in a single molecule. This approach may improve antitumour activity, reduce total drug load and minimize undesired drug–drug interactions. A representative example of this strategy is NVP-AEE788 (**3**, Fig. 1) [12], an EGFR/erbB2/VEGFR inhibitor that can target simultaneously tumour cell proliferation and vascularisation. In the same direction, the potential issue of resistance due to mutations in the targeted kinase or the presence of compensatory signal transduction pathways may also require the identification of single-agents with spectrum selective inhibitory activity.

The use of structural information by X-ray crystallography or computer-assisted molecular modelling based on kinase domain homology has played a major role in the identification by structure-based design approaches of the compounds currently undergoing clinical trials [13]. The growing body of structural data for kinases [14] and the implementation of a new set of structure-based screening technologies [15] seem to offer attractive prospects in this area of research. Representative examples of the new ways that structural biology can contribute to the identification and development of the next generation of antikinase cancer agents are targeting the inactive form of the kinase [16], understanding the mechanism of resistance mutations [17] or short-cutting the process of hit identification and hit-to-lead optimisation [15].

As an alternative to ATP site-directed inhibitors, antibody-based approaches and growth factor antagonists have been explored to inhibit receptor tyrosine kinases [18]. In this case, members of this subclass of transmembrane-spanning proteins have been inhibited by blocking the physical interaction of the extracellular domain of the receptor with its cognate ligand. Contrary to the successful development of humanized monoclonal antibodies against EGFR, erbB2 or VEGFR [19][20], the identification of low-molecular mass antagonists of growth factors has been mainly restricted to peptide-like molecules [21–23]. Although these peptides have displayed a diverse range of biological activities both *in vitro* and *in vivo*, their use as drugs can be compromised by proteolytic degradation, rapid elimination from plasma, high first-pass metabolism, and low oral bioavailability. Structure-based design approaches that build upon three-dimensional models of the growth factor/receptor complex interactions [25], and new methods to increase compound diversity [26][27] may eventually identify and optimise non-peptidic antagonists for this relatively unexplored, but attractive, drug discovery strategy for oncogenic receptor tyrosine kinases.

3. ATPases: Heat-Shock Protein 90 and Kinesin Spindle Protein

ATPases are enzymes that use the energy originating from the hydrolysis of ATP to drive thermodynamically unfavourable biological processes such as protein folding, intracellular transport, protein degradation or ion transport. Several members of this class of enzymes have recently emerged as attractive targets in oncology and major drug discovery advances have been accomplished for two of them: heat-shock protein 90 (Hsp90) and kinesin spindle protein [28][29].

Hsp90 is a molecular chaperone involved in the folding and stability of a selected range of client proteins. This chaperone, which is over-expressed in certain types of tumours, is believed to be involved in dealing with the cellular stress associated with the hostile cancer environment, as well as being essential for the proper function of key oncogenic proteins (*e.g.* erbB2, c-Raf, mutated p53, PKB/Akt). The association with Hsp90 allows these proteins to be operational in signal transduction pathways that are essential to mediate and sustain tumour cell growth and survival. Hence, inhibitors of Hsp90 may block a wide range of 'cancer hallmark traits' exhibiting a broad-spectrum antitumour activity across multiple cancer types [30][31].

In terms of development of Hsp90 inhibitors as anticancer agents, initial attention was focused on two natural products, geldanamycin (**4a**, Fig. 2) and radicicol (**5**, Fig. 2) [26]. X-ray crystallographic studies revealed that these compounds bind into the ATP-binding cleft of the N-terminal domain of Hsp90. Occupancy of this pocket results in inhibition of the essential ATPase activity that drives the molecular engine of this chaperone. In preclinical studies, these initial Hsp90 inhibitors showed potent antiproliferative activity in cellular settings and *in vivo* antitumour activity in a variety of human tumour xenografts [32]. One of these derivatives, 17-allylamino-17-demethoxygeldanamycin (17-AAG; **4b**, Fig. 2), is currently in Phase II clinical trials. Although 17-AAG has shown some encouraging clinical responses, it presents important drawbacks (*e.g.* formulability, hepatotoxicity) that may hamper its full development. To address these shortcomings, geldanamycin derivatives with improved pharmaceutical properties have been prepared and one derivative 17-

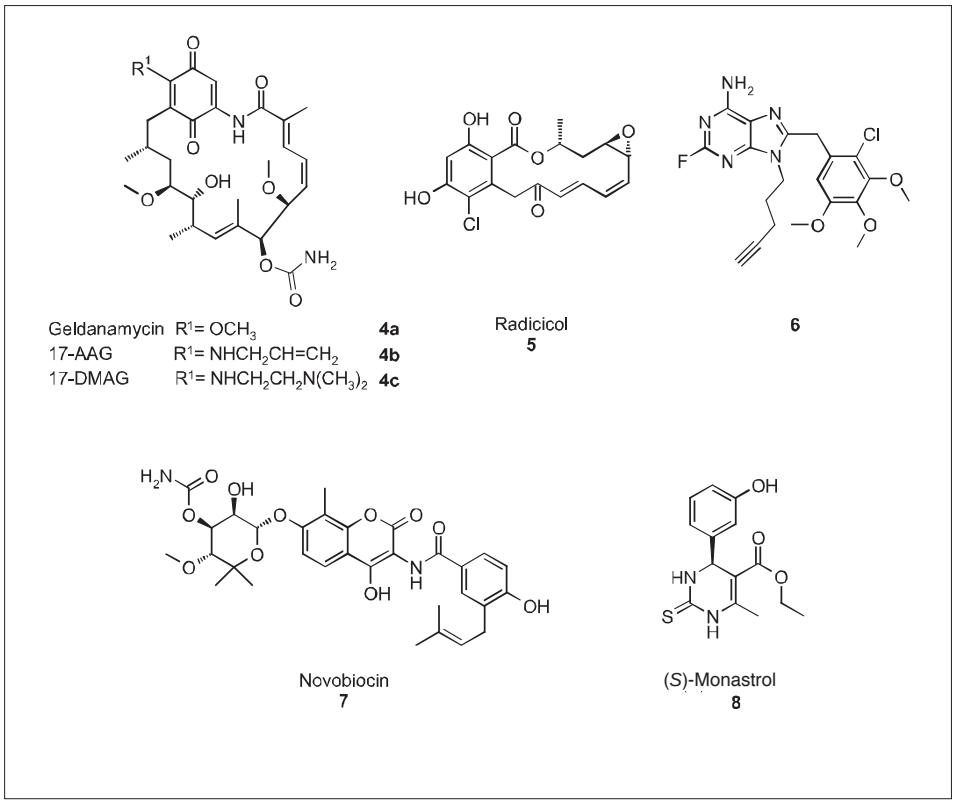

Fig. 2. Representative examples of inhibitors of Hsp90 and KSP.

desmethoxy-17-N,N-dimethylaminoethylamino-geldanamycin (17-DMAG; **4c**, Fig. 2) has recently entered Phase I clinical trials.

The preclinical data and the tumour selectivity [33] observed with the initial Hsp90 inhibitors have spurred a widespread interest in the identification of new chemotypes that interfere with ATP binding [34]. Structure-based design and high-throughput screening approaches have been taken to meet this objective, but so far the results have been daunting. The most relevant low-molecular-mass Hsp90 inhibitors described to date are purine-based compounds [35]. Although some of these compounds are equipotent to 17-AAG in biochemical assays (*e.g.* **6**, Fig. 2), the specificity and *in vivo* antitumour activity of these purines have not yet been reported.

In addition to the ATP-binding pocket at the N-terminus of Hsp90, a recent study has suggested a potential ATP binding cleft at the C-terminus of this chaperone [36]. Occupancy of this pocket by novobiocin (**7**, Fig. 2), which is an antibiotic produced by the actinomycete *Streptomyces nivens* and used to treat infections by gram-positive bacteria, impairs the cellular function of Hsp90. This finding may offer a new avenue to modulate Hsp90 activity by targeting an alternative nucleotide-binding site with different structural features [29].

The other emerging therapeutic target from this nucleotide-binding family is the kinesin spindle protein (KSP) [37]. This ATPase, which is a member of the kinesin subfamily of microtubule molecular motors, converts the energy released from the hydrolysis of ATP molecules into mechanical force that drives the transport of cellular cargoes along microtubules [38]. A functional KSP is required for the establishment and maintenance of bipolar spindle formation during mitosis, and the proper segregation of replicated DNA into the two daughter cells [39]. According to its critical role in dividing cells, perturbation of KSP activity in proliferating tumour cells could cause malformation or dysfunction of the mitotic spindle and induction of cell cycle arrest and apoptosis.

KSP consists of an N-terminal motor head, a central coiled coil, and a C-terminal tail. Inhibition of KSP activity has been accomplished by targeting the ATP binding cleft in the motor domain. Quinazolines, isoquinolinones, dihydroindolylcarboxylates, pyrido-pyrimidines, pyrimidinones, pyrazoles, thienopyrimidines, phenothiazines, and triphenylmethanes have been claimed in different patents as KSP inhibitors. Although we can assume that some of these compounds exert their inhibitory activity by interfering with ATP-binding [28][40], a recent article has reported a potential alternative mechanism for blocking KSP. The X-ray structure of KSP complexed with (*S*)-monastrol (**8**, Fig. 2) and Mg^{2+}·ADP revealed that this inhibitor binds to an induced-fit pocket 12 Å away from the nucleotide-binding cleft of the protein [42]. The interaction of monastrol with the protein induced conformational rearrangements throughout the motor domain that may eventually affect the *in vivo* movement of KSP on the microtubule.

The paucity of published data on the *in vitro* and *in vivo* antitumour activities of KSP inhibitors make it difficult to assess at this moment the potential benefit and therapeutic window of this new type of antimitotic agent. An important preclinical observation is that unlike taxanes, SB 715992 (CK 0238273; structure not disclosed), which is a KSP inhibitor in Phase II clinical trials, did not cause peripheral neuropathy in a mouse model [43]. This is in alignment with the hypothesis that KSP is essential for assembly and function of the mitotic spindle in proliferating cells, but it is not involved in the microtubule dynamics associated with some nerve processes.

4. Histone Deacetylase Inhibitors

Aberrant regulation of gene expression is a hallmark of cancer cells and reversal of this effect has been considered a sound strategy for cancer therapy. Among the proteins involved in these epigenetic events, histone deacetylases (HDACs) have been the subject of intense drug discovery efforts. Together with histone acetyltransferases, HDACs play an important role in modulating the topology of chromatin and regulating gene expression. Inappropriate HDAC-mediated transcriptional repression in tumour cells produces alterations in chromatin structure and blockage of cell differentiation [44]. Consistent with this biological process, inhibition of HDACs and activation/repression of specific genes may result in cell cycle arrest, stimulation of differentiation, and induction of apoptosis [45].

Current HDAC inhibitors can be divided into five broad structural classes: hydroxamic acids (*e.g.* **9**, Fig. 3), aliphatic acids or pro-drugs thereof (*e.g.* **10**, Fig. 3), benzamides (*e.g.* **11**, Fig. 3), electrophilic ketones (*e.g.* **12**, Fig. 3), and cyclic peptides (*e.g.* **13** and **14**, Fig. 3) [46][48][49]. Overall and with a few exceptions, these chemotypes conform to a general pharmacophore that is composed of three main structural elements: i) a zinc interacting group; ii) a linear or conformationally constrained spacer; and iii) a surface recognition motif that interacts with amino acids on the rim of the active site. Together with homology models and ex-

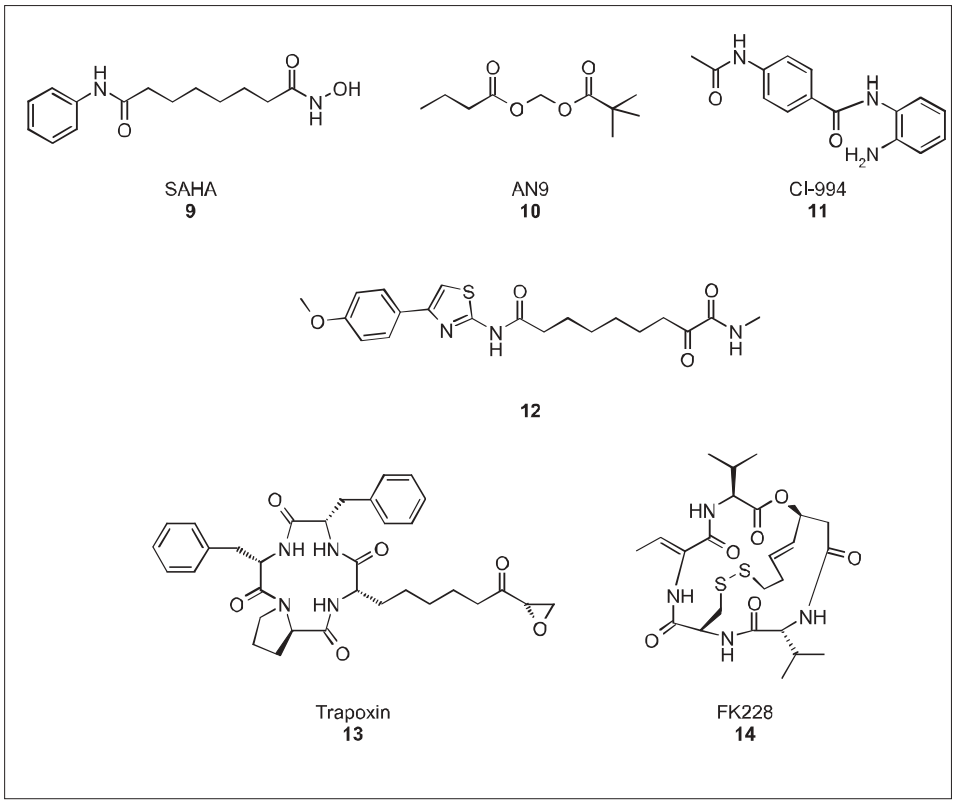

Fig. 3. Representative examples of histone deacetylase inhibitors.

tensive SAR studies, the structural details of HDAC inhibitor-enzyme interactions obtained by X-ray crystallography have been instrumental in the identification and development of therapeutic agents in this area of research. Representative examples of HDAC inhibitors in clinical trials are shown in Fig. 3 (**9**–**11**, **14**).

In preclinical models, HDAC inhibitors have shown potent antiproliferative activities against a broad spectrum of transformed cells, and *in vivo* antitumour activities in xenograft models without apparent toxicity [49]. These inhibitory effects are believed to be caused in part by accumulation of acetylated proteins, such as nucleosomal histones, and activation/repression of a small number of genes (*e.g.* activation of p21). However, the greater inhibitory activity on transformed cells compared with normal cells upon incubation with HDAC inhibitors in culture are not completely understood.

Phase I/II clinical trials with HDAC inhibitors either as single agent or in combination studies are ongoing. The compounds seem to be well tolerated, and stable diseases or mild improvements in cancer patients have been reported for some of them [47], but additional results will be required to ultimately determine the clinical utility of these agents.

The ability to selectivity inhibit specific HDACs is currently a major focus in this area of research. Constituents of the hydroxamic acid class and some of the cyclic peptides are among the most potent HDAC inhibitors reported to date, but the intrinsic features of these inhibitors (*e.g.* strong interaction with zinc or tight contacts in the active site) make them non-selective against the different members of the class I/II HDACs. It is reasonable to predict that the use of structure-based design approaches will have the greatest opportunity to modulate the selectivity profile of the current scaffolds or identify new lead structures. In this context, exploiting the structural diversity on the periphery of the N-acetyl lysine binding channel and fine-tuning the interactions mediated by the surface recognition domain have provided initial hints on how to achieve some differential selectivity [51–53].

5. Conclusions

As illustrated with the three target family briefly reviewed in the preceding sections, the current challenge in oncology drug discovery is to identify molecules able to block specific biochemical processes that are considered essential for the malignant phenotype of cancer cells. Meeting this challenge is likely to produce therapies with a level of efficacy and therapeutic index not possible with existing anticancer agents. Looking at the results obtained in the past few years, it is remarkable to see how multidisciplinary teams have been able to block oncogenic targets in ways not previously possible. As these targeted therapies move forward in the development path, we can only hope that some of the promising findings observed in preclinical models with these new anticancer agents may eventually come to be realized in clinical settings.

Acknowledgements

Thanks go to S.C. DiClemente, P. Holzer, and F. Stauffer for their comments and suggestions during the elaboration of this article.

Received: June 18, 2004

[1] R. Abou-Jawde, T. Choueiri, C. Alemany, T. Mekhail, *Clin. Ther.* **2003**, *25*, 2121.
[2] N.-H. Nam, K. Parang, *Curr. Drug Targets* **2003**, *4*, 159.
[3] G. Gasparini, M. Gion, *Cancer J.* **2000**, *6*, 117.
[4] J. Dancey, E.A. Sausville, *Nature Rev.* **2003**, *2*, 296.
[5] P. Traxler, *Expert Opin Ther Targets* **2003**, *7*, 215.
[6] F. de Bree, L.A. Sorbera, R. Fernandez, J. Castaner, *Drugs* **2001**, *26*, 545.
[7] R.S. Herbst, *Expert Opin. Invest. Drugs* **2002**, *11*, 837.
[8] B.J. Druker, *Trends Mol. Med.* **2002**, *8*, S14.
[9] J. Zimmermann, E. Buchdunger, H. Mett, T. Meyer, N.B. Lydon, P. Traxler, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1221.
[10] E. Buchdunger, J. Zimmermann, H. Mett, T. Meyer, M. Mueller, B.J. Druker, N.B. Lydon, *Cancer Res.* **1996**, *56*, 100.
[11] D.G. Savage, K.H. Antman, *N. Engl. J. Med.* **2002**, *346*, 683.
[12] G. Caravatti, B. Guido, J. Brueggen, P. Furet, H. Lane, J. Mestan, T. Meyer, C. Tang, M. Wartmann, J. Wood, A. Zimmerlin, P. Traxler, *Proc. AACR-NCI-EORTC Int. Conf.* **2003**, abstract 118.
[13] M.E.M. Noble, J.A. Endicott, L.N. Johnson, *Science* **2004**, *303*, 1800.
[14] X-ray crystal structures have been reported to date for around 50 protein kinases. This value is still relatively low because it represents less than 10% of the estimated number of protein kinases in the human genome. For additional information on this topic, the reader is directed to *http://www.sdsc.edu/kinases*, which covers a comprehensive list of protein kinases including 3D structural information.
[15] A. Gill, *Mini Rev. Med. Chem.* **2004**, *4*, 301.
[16] T. Schindler, W. Bornmann, P. Pellicena, W.T. Miller, B. Clarkson, J. Kuriyan, *Science* **2000**, *289*, 1938.
[17] S.W. Cowan-Jacob, V. Guez, G. Fendrich, J.D. Griffin, D. Fabbro, P. Furet, J. Liebetanz, J. Mestan, P.W. Manley, *Mini Rev. Med. Chem.* **2004**, *4*, 285.
[18] D. Fabbro, C. Garcia-Echeverria, *Curr. Opin. Drug Discov. Devel.* **2002**, *5*, 701.

[19] C. Arteaga, *Semin. Oncol.* **2003**, *30*, 3.
[20] N.Z. Anon, *Drugs R.D.* **2002**, *3*, 28.
[21] M.A. Blaskovich, Q. Lin, F. Delarue, J. Sun, H.S. Park, D. Coppola, A.D. Hamilton, S.M. Sebti, *Nature Biotech.* **2000**, *18*, 1065.
[22] P. Häyry, M. Myllärniemi, E. Aavik, S. Alatalo, P. Aho, S. Yilmaz, A. Räisänen-Sokolowski, G. Cozzone, B.A. Jameson, R. Baserga, *FASEB J.* **1995**, *9*,1336.
[23] In addition to these peptide-like molecules, suramin, which is a polyanionic naphthlyurea derivative, blocks the interaction of a variety of growth factors to their receptor tyrosine kinases [24].
[24] M.C. Russell, H. Mach, C.J. Burke, D.A. Volkin, J.M. Dabora, P.K. Tsai, M.W. Bruner, J.A. Ryan, K.E. Marfia, *Biochemistry* **1992**, *31*, 9016.
[25] C. Tan, L. Wei, F.P. Ottensmeyer, I. Goldfine, B.A. Maddux, C.C. Yip, R.A. Batey, L.P. Kotra, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1407.
[26] M. Rarey, M. Stahl, *J. Comput. Aided Mol. Des.* **2001**, *15*, 497.
[27] L. Xue, J.W. Godden, F.L. Stahura, J. Bajorath, *Comput. Aided Drug Discov.* **2001**, *7*, 125.
[28] Z. Gao, D.M. Duhl, S.D. Harrison, *Annu. Rep. Med. Chem.* **2003**, *38*, 193.
[29] P. Chène, *Nature Rev.* **2002**, *1*, 665.
[30] P. Workman, *Cancer Lett.* **2004**, *206*, 149.
[31] P. Workman, *Curr. Cancer Drug Targets* **2003**, *3*, 297.
[32] L. Neckers, *Trends Mol. Med.* **2002**, *8*, S55.
[33] A. Kamal, L. Thao, J. Sensinataffar, L. Zhang, M.F. Boehm, L.C. Fritz, F.J. Burrows, *Nature* **2003**, *425*, 407.
[34] L. Neckers, *Curr. Med. Chem.* **2003**, *10*, 733.
[35] G. Chiosis, B. Lucas, A. Shtil, H. Huezo, N. Rosen, *Bioorg. Med. Chem. Lett.* **2003**, *10*, 3555.
[36] M.G. Marcu, A. Chadli, I. Bouhouche, M. Catelli, L.M. Neckers, *J. Biol. Chem.* **2000**, *275*, 37181.
[37] T.U. Mayer, T.M. Kapoor, S.J. Haggarty, R.W. King, S.L. Schreiber, T.J. Mitchison, *Science* **1999**, *286*, 971.
[38] R.D. Vale, R.A. Milligan, *Science* **2000**, *288*, 88.
[39] A. Blangy, H.A. Lane, P. d'Herlin, M. Harper, M. Kress, E.A. Nigg, *Cell* **1995**, *83*, 1159.
[40] No ATP-competitive inhibitors for KSP have been described, see [41].
[41] S.C. Hopkins, R.D. Vale, I.D. Kuntz, *Biochemistry* **2000**, *39*, 2805.
[42] Y. Yan, V. Sardana, B. Xu, C. Homnick, W. Halczenko, C.A. Buser, M. Schaber, G.D. Hartman, H.E. Huber, L.C. Kuo, *J. Mol. Biol.* **2004**, *335*, 547.
[43] R.K. Johnson, F.L. McCabe, E. Caulder, L. Inlow-Porter, M. Whitacre, J.D. Winkler, G. Bergnes, B. Feng, D. Morgans, K.W. Wood, J.R. Jackson, *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, abstract 1335.
[44] P. Jones, S.B. Baylin, *Nat. Rev. Genet.* **2002**, *3*, 415.
[45] R.R. Rosato, S. Grant, *Cancer Biol. Ther.* **2003**, *2*, 30.
[46] Cyclic peptides that inhibit HDAC have been classified in two general groups [47]: inhibitors bearing the amino acid (*S*)-2-amino-9,10-8-oxodecanoic acid (L-Aoe, *e.g.* **13**, Fig. 4), and peptides without this epoxy ketone (*e.g.* **14**, Fig. 4). The mechanism of action of L-Aoe containing peptides has not been fully clarified. Irreversible inhibition or slow dissociation have been suggested as potential mechanistic possibilities to explain the inhibitory activity displayed by these HDAC inhibitors.
[47] P.A. Marks, T. Miller, V.M. Ricohn, *Curr. Opin. Pharmacol.* **2003**, *3*, 351.
[48] T.A. Miller, D.J. Witter, S. Belvedere, *J. Med. Chem.* **2003**, *46*, 5097.
[49] W.K. Kelly, O.A. O'Connor, P.A. Marks, *Drugs* **2002**, *11*, 1695.
[50] Class I/II HDACs, which are the major targets for cancer treatment, operate by a zinc-dependent mechanism. HDACs **1**, **2**, **3** and **8** are members of class I and they share high homology in their catalytic site. Class II is formed by HDACs **4**, **5**, **6**, **7**, **9** and **10**.
[51] S.M. Sternson, J.C. Wong, C.M. Grozinger, S.L. Schreiber, *Org. Lett.* **2001**, *3*, 4239.
[52] M. Yoshida, R. Furumai, M. Nishiyama, Y. Komatsu, N. Nishino, S.M. Horinouchi, *Cancer Chemother. Pharmacol.* **2001**, *48*, S20.
[53] R. Furumai, Y. Komatsu, N. Nishino, S. Khochbin, M. Yoshida, S. Horinouchi, *Proc. Natl. Acad. Sci. USA* **2002,** *98,* 87.