Recent Advances in the Development of Multi-Kinase Inhibitors

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Abstract: During the last two decades, protein kinases have emerged as a major target for cancer therapy and a large number of selective kinase inhibitors have been developed as potential anticancer drugs. To avoid unpredictable toxic effects, researchers usually aim at designing highly selective inhibitors. But since the formation and progression of a tumor has to be considered as a multifactorial process, which is dependent on different signalling pathways, it seems reasonable to establish anticancer therapies that target several kinases associated with tumor growth. In general, this can be achieved by two different strategies, either by concomitantly using a combination of a set of selective kinase inhibitors or by administering a single agent, which simultaneously inhibits several kinases, a so called multi-kinase inhibitor. In this review, benefits and obstacles of both strategies are discussed. An overview over recently approved and newly upcoming multi-kinase inhibitors is given.

Key Words: Multiple targeted, kinase inhibitor, development.

[#]**Author Profile:** Andreas Hilgeroth studied pharmacy in Düsseldorf and received his PhD with Professor Uwe Kuckländer in 1994. After a post-doc period, he received a fellowship from the German Pharmaceutical Society (DPhG) to work at the institute of pharmaceutical chemistry at the Martin-Luther University in Halle-Wittenberg, where he received his habilitation in 2000. Since then he has been a research group leader and develops novel antiretroviral drugs, potential alzheimer therapeutics and multi-kinase inhibitors. He received several prizes for outcoming advances in solid-state chemistry and multidrug resistance (MDR) drug developments.

INTRODUCTION

The reversible phosphorylation of proteins by protein kinases is one of the most important regulatory mechanisms and plays a pivotal role in processes like the transduction of external signals and the cell cycle regulation. Therefore, protein kinases have emerged as major targets for drug discovery, especially for the developement of novel anticancer drugs. In 2002, it was estimated that protein kinases account for about 20-30% of the R & D budget of the pharmaceutical industry, making them the second most investigated targets after the GPCRs (G-protein coupled receptors) [1]. The vast majority of inhibitors of protein kinases are small heterocyclic compounds which are directed against the catalytic site of a kinase and inhibit the enzyme in an ATP-(adenosine triphosphate)-competitive way. Due to the ubiquitous nature of ATP as the common substrate of all kinases and the generally high degree of conservation of the amino acid residues which form the ATP binding site throughout all protein kinases families, an ATP-competitive inhibitor normally does not selectively inhibit only one kinase, but a more or less defined set of structurally related kinases (then, it is called a "multi-kinase inhibitor") or even a broad spectrum of kinases (then, it is often called a "pan-kinase inhibitor"). To avoid toxic effects that are caused by the inhibition of other kinases than the intended one, researchers usually aim at designing inhibitors which are highly selective for a single kinase. However, clinical experience has told us that for the treatment of complex diseases, "dirty drugs" which simultaneously hit multiple targets are often more effective than single protein targeted compounds and often have less unwanted side effects than is generally assumed, as it is the case with many older and well established compounds like aspirin or the multi-receptor targeted antipsychotic drug clozapine [2]. Since the formation and progression of a tumor have to be considered as multistep processes that needs to be stimulated and maintained by different signalling pathways, it appears reasonable to simultaneously address several of these pathways. In general, the inhibition of a more or less defined set of protein kinases can be achieved either by using a single agent which shows activity against these kinases or by using a combination of several selective kinase inhibitors. Both of these strategies might have certain advantages and disadvantages: Taking a single, multi-kinase targeted drug might be easier to conduct but is less flexible. Rather than inhibiting all kinases of interest with the same potency (that is, with the same IC_{50}-values), a typical multi-kinase inhibitor possesses a certain potency spectrum that cannot be changed. This might constrain its therapeutic use to certain defined cases, for example particular tumor types which possess a pattern of protein kinase overexpression that exactly corresponds to the inhibition profile of the multi-kinase inhibitor. Otherwise, if a combination of several single kinase inhibitors is used instead, the inhibition profile can in theory be adjusted to perfectly meet the therapeutic requirements, but as is true for all combinations of therapeutic agents, drug-drug interactions might occur and adversely affect absorption, metabolism, and excretion of the agents and there-

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fore change plasma levels of the compounds and thus activities against particular kinases. If one agent inhibits membrane efflux pumps like P-glycoprotein (P-gp) or the breast cancer resistance protein (BCRP), the intracellular concentration of a second, simultaneously administered agent might easily reach toxic levels. The same is true for interactions with drug metabolizing enzymes, e. g. cytochrome-P-450. These problems will not occur if a single drug is used to address the targets of interest. Finally, it has to be mentioned that the rational development of clinically useful and not merely nonselective and therefore unsafe "dirty drugs" appears to be much more challenging than creating a single protein targeted agent. Performing an efficient screening for a multi-targeted drug is certainly much more difficult than identifying the best binder for a single protein, especially if the optimal candidate might be a compound that binds to its targets with comparable low affinities. Furthermore, the clinical effects and unwanted side effects of a multi-targeted drug will be more complex, harder to predict and to influence. This can impede several steps of drug development, as for instance optimization of the chemical structure, drug delivery, and dose regimes. Surely, it can be speculated that most or even all of the multi-kinase inhibitors which are currently in the "pipeline" of the pharmaceutical industry were initially designed to be and developed as selective for a single kinase and later turned out to inhibit other kinases as well. Furthermore, it seems reasonable to assume that many compounds which are currently published to be highly selective kinase inhibitors would reveal additional activities if tested against a broad panel of different kinases.

The aim of this review is to give an overview over the multi-kinase inhibitors that already have received approval for the treamtent of cancer and to provide information on the most important advanced drug candidates in this field. All approved multi-kinase inhibitors and the most intensively studied drug candidates along with their alternative names, their targets, their manufacturer, and their current developmental status are listed in Table **1**.

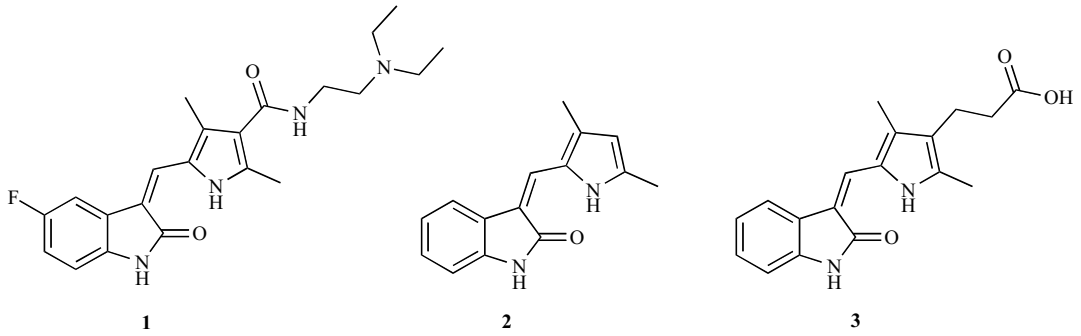
INDOLINE-2-ONES

3-Substituted indoline-2-ones were among the first structures which were evaluated as kinase inhibitors and have been intensively studied. They were initially developed as VEGFR-(vascular endothelian growth factor receptor)-selective, antiangiogenic agents, but soon after their discovery, it was found that they also inhibit a set of other, structurally related kinases, including c-Kit (the protein product

Table 1. Currently Approved Multi-Kinase Inhibitors and Advanced Drug Candidates

Substance Class	Name	Other Names	Target Kinases	Manufacturer	Status
indoline-2-ones	Sunitinib	Sutent, SU11248	VEGFR 1-3[a], c-Kit[b], Flt-3[c], PDGFR-α/β[d], CSF-1-R[e]	Pfizer	approved
indazoles	Axitinib	AG-013736	VEGFR-1-3[a], PDGFR[d]	Pfizer	phase III
indazoles	Pazopanib	GW-786034	VEGFR-1-3[a]	GlaxoSmithKline	phase III
phthalazines and related compounds	Vatalanib	PTK/ZK787	VEGFR-1-3[a], PDGFR[d]	Novartis	phase II
	Telatinib	BAY 57-9352	VEGFR-2/3[a], PDGFR-β[d], c-Kit[b]	Bayer-Schering	phase Ib
	Motesanib	AMG-706	VEGFR 1-3[a], PDGFR[d], c-Kit[b]	Amgen	phase II
quinazolines	Lapatinib	Tykerb, GW572016	EGFR[f], HER2[g]	GlaxoSmithKline	approved
	Vandetanib	Zactima, ZD-6474	EGFR[f], VEGFR-1[a], RET[h], Abl[i]	AstraZeneca	phase II
	Tandutinib	MLN518	PDGFR-β[d], Flt-3[c], CSF-1-R[e], c-Kit[b]	Millennium	phase II
	AZD0530	none	c-Src[j], Abl[i]	AstraZeneca	phase II
	Canertinib	CI-1033, PD183805	EGFR[f], HER2[g]	Pfizer	phase II
other fused pyrimidines	BMS-599626	none	EGFR[f], HER2[g]	Bristol-Myers Squibb	phase III
	AEE788	NVP-AEE788	EGFR[f], HER2[g], VEGFR-2[a]	Novartis	phase II
benzamides	Imatinib	Gleevec, STI-571	Bcr-Abl[k], PDGFR-α/β[d], c-Kit[b]	Novartis	approved
	Dasatinib	BMS-354825	Bcr-Abl[k], PDGFR-α/β[d], c-Kit[b]	Bristol-Myers Squibb	approved
	Nilotinib	Tasigna, AMN107	Bcr-Abl[k], PDGFR-α/β[d], c-Kit[b]	Novartis	approved
	INNO-406	NS-187	Bcr-Abl[k], PDGFR-α/β[d], c-Kit[b]	Innovive	phase I
diphenylureas	Sorafenib	Nexavar, BAY43-9006	Raf-1[l], VEGFR-1-3[a], Flt-3[c], c-Kit[b], PDGFR-β[d]	Bayer-Schering	approved

[a]VEGFR: vascular endothelian growth factor receptor; [b]Flt-3: FMS-like tyrosine kinase 3; [c]c-Kit: protein product of the proto-oncogene c-Kit; [d]PDGFR: platelet derived growth factor receptor; [e]CSF-1-R: colony stimulating factor 1 receptor; [f]EGFR: epidermal growth factor receptor; [g]HER2: human epidermal growth receptor 2; [h]RET: protein product of the RET ("rearranged during transfection") proto-oncogene; [i]Abl: Abelson kinase; [j]c-Src: protein product of the proto-oncogene; [k]Bcr-Abl: protein product of the Bcr-Abl fusion gene; [l]Raf-1: protein product of the proto-oncogene Raf-1.



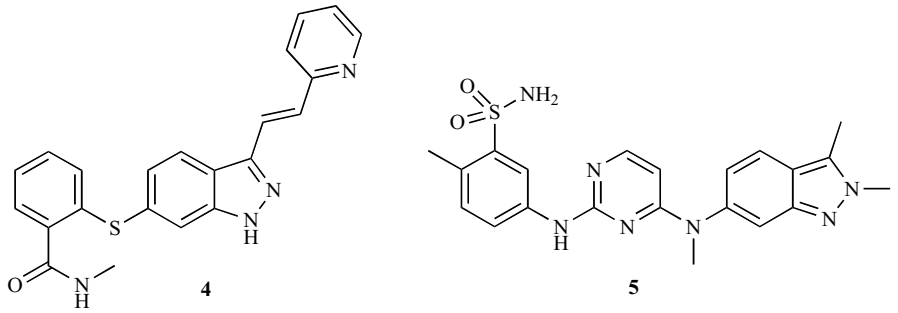
1 **2** **3**

Fig. (1).

of the proto-oncogene Kit), Flt-3 (FMS-like tyrosine kinase 3), PDGFR-α/β (platelet derived growth factor receptor), and CSF-1-R (colony stimulating factor 1 receptor), rather than being specific for VEGFR 1-3. However, since the indolin-2-ones are remarkably small agents, which exclusively occupy the ATP binding pocket without interacting with peripheral, more kinase-specific areas of the enzyme catalytic site, it is quite surprising that no inhibition of other, unrelated kinases has ever been reported in the literature. The first multi-targeted kinase inhibitor of the indolin-2-one type that reached the clinic is Sunitinib **1** (Sutent®, SU11248, Pfizer) [3]. In January 2006, Sunitinib was approved by the FDA (Food and Drug Administration) for the treatment of advanced RCC (renal cell carcinoma) and GIST (gastrointestinal stroma tumors) in patients, who do not respond to or do not tolerate Imatinib. Sunitinib is the first anticancer drug which received simultaneous FDA-approval for two different indications. In preceding clinical studies, Sunitinib was shown to be effective in the treatment of Imatinib-resistant GIST, leading to an elongation of the median time to tumor progression to 27.3 weeks in patients receiving Sunitinib compared to 6.4 weeks in those receiving placebo [4], and also in the treatment of RCC, where the median progression-free survival time was enlarged to 11 months in the Sunitinib group compared to 5 months in a group of patients receiving interferon α [5]. In both cases, Sunitinib was relatively well tolerated, with only few and generally mild side effects. Due to its impressing effect and its favourable safety profile, the use of Sunitinib is currently evaluated for the treatment of a large number of other malignancies [6]. Recently published data from ongoing clinical studies indicate that Sunitinib also shows activity in the treatment of metastatic breast cancer [7], NSCLC (non small cell lung cancer) [8], and unresectable neuroendocrine tumors [9]. In contrast to the successful introduction of Sunitinib, the results obtained with two other widely studied indoline-2-ones, Semaxanib **2** (SU5416) and SU0006668 **3** (also called SU6668 or TSU-68), were disappointing: In a phase II study conducted with thirteen patients with advanced soft tissue sarcomas, Semaxanib was shown to be well tolerated but failed to show any significant antitumor effect. The ineffectiveness of Semaxanib was ascribed to a lack of inhibition of c-Kit and VEGFR *in vivo* observed during the conduction of this trial [10]. For the other indolin-2-one derivative which is frequently mentioned in the literature, SU000668, data from two phase I studies were recently published. In both cases, SU000668 was found to possess unfavourable pharmacokinetic properties, characterized by induction of metabolism after chronic oral dosing, high plasma protein binding and low achievable plasma levels [11].

INDAZOLES

The substituted indazole derivative Axitinib **4** (AG-013736, Pfizer) is a potent inhibitor of all three VEGFRs, which are inhibited with IC_{50}-values in the subnanomolar range, and of PDGFR-β and c-Kit, which are inhibited at low nanomolar concentrations. In mice, it is able to inhibit tumor vascular angiogenesis and the growth of human colorectal, breast, and murine lung tumors [12], and to induce tumor regression in Bevacizumab-resistant human colon carcinoma and melanoma xenograft tumors [13]. A second drug candidate of the indazole class is Pazopanib **5** (GW-786034**,** GlaxoSmithKline), which inhibits VEGFR-1-3 with IC_{50}-values of 10, 30, and 47 nM, respectively. Additionally, it shows activity against PDGFR-α/β and c-Kit. In an initial non randomized, dose-escalation phase I study in patients with refractive RCC or other malignancies, including GIST and melanoma, Pazopanib was shown to be well tolerated,



4 **5**

Fig. (2).

with hypertension, fatigue, and hair depigmentation being the most frequently observed side effects. Out of twelve patients with RCC, seven achieved a stable disease or tumor reduction, and one patient showed a partial response [14]. Currently, Pazopanib is evaluated for the treatment of metastatic RCC in a phase III clinical trial.

PHTHALAZINES AND COMPOUNDS DERIVED THEREOF

In 2000, the disubstituted pthalazine Vatalanib **6** (PTK/ZK787, PTK787/ZK222584, and PTK/ZK) was discovered by screening a Novartis corporate library for VEGFR-inhibitors [15]. The lead compound Vatalanib was shown not only to potently inhibit VEGFR-1 (IC_{50}: 54 nM), -2 (39 nM) -3 (30 nM), but also other kinases of the VEGFR-family, notably PDGFR-β (567 nM), c-Kit (600 nM), and CFMS-1-R (600 nM). However, Vatalanib did not inhibit any kinases of other families - except of Lyn (IC_{50}: 1.8 μM) and c-raf (IC_{50}: 3.6 μM) [16], but the structural background of this selectivity remained unclear. As Vatalanib was found to be orally active and was generally well tolerated, it entered further evaluations without structural optimization. In preclinical tests, Vatalanib inhibited both angiogensis and tumor growth in a number of tumor models [17]. Recently, the efficacy of Vatalanib in the treatment of metastatic colorectal cancer was evaluated in two randomized, double-blind phase III studies, CONFIRM-1 and -2. In both studies the effect of the addition of Vatalanib to a conventional FOLFOX-4-therapy (a combination therapy containing fluorouracil, oxaliplatin, and folinic acid) was investigated. In CONFIRM-1, the addition of Vatalanib did neither significantly affect the response rate, nor the progression free survival time. In CONFIRM-2, the overall survival was only sligthly higher under Vatalanib, whereas the progression free time was significantly longer in the Vatalanib arm [18]. It is assumed that the mainly disappointing results of these study are at least partly due to a suboptimal dosing of Vatalanib, as the agent has a relatively short half life and it has been suggested to dose the compound twice daily to obtain better results [19]. For several years, Vatalanib remained to be the only multi-kinase inhibitor of the phtalazine type and little was known about its binding mode and structure activity relationships, but during the last two years, a large number of derivatives of Vatalanib have been published: For instance, it was shown that the picolyl substituent of Vatalanib can be replaced by benzamides and benzoic acid esters like in **7** [20], but also by a phenyl ring bearing an imidazole, pyrazole, triazole, or tetrazole ring in its 4-position, as it is exemplified by compound **8**, or even by fused nitrogen-containing bicycles, as for example **9** [21], which indicates that the nature of this substituent is irrelevant as long as it contains a hydrogen bond donor within a certain distance away from the core structure. Addi-

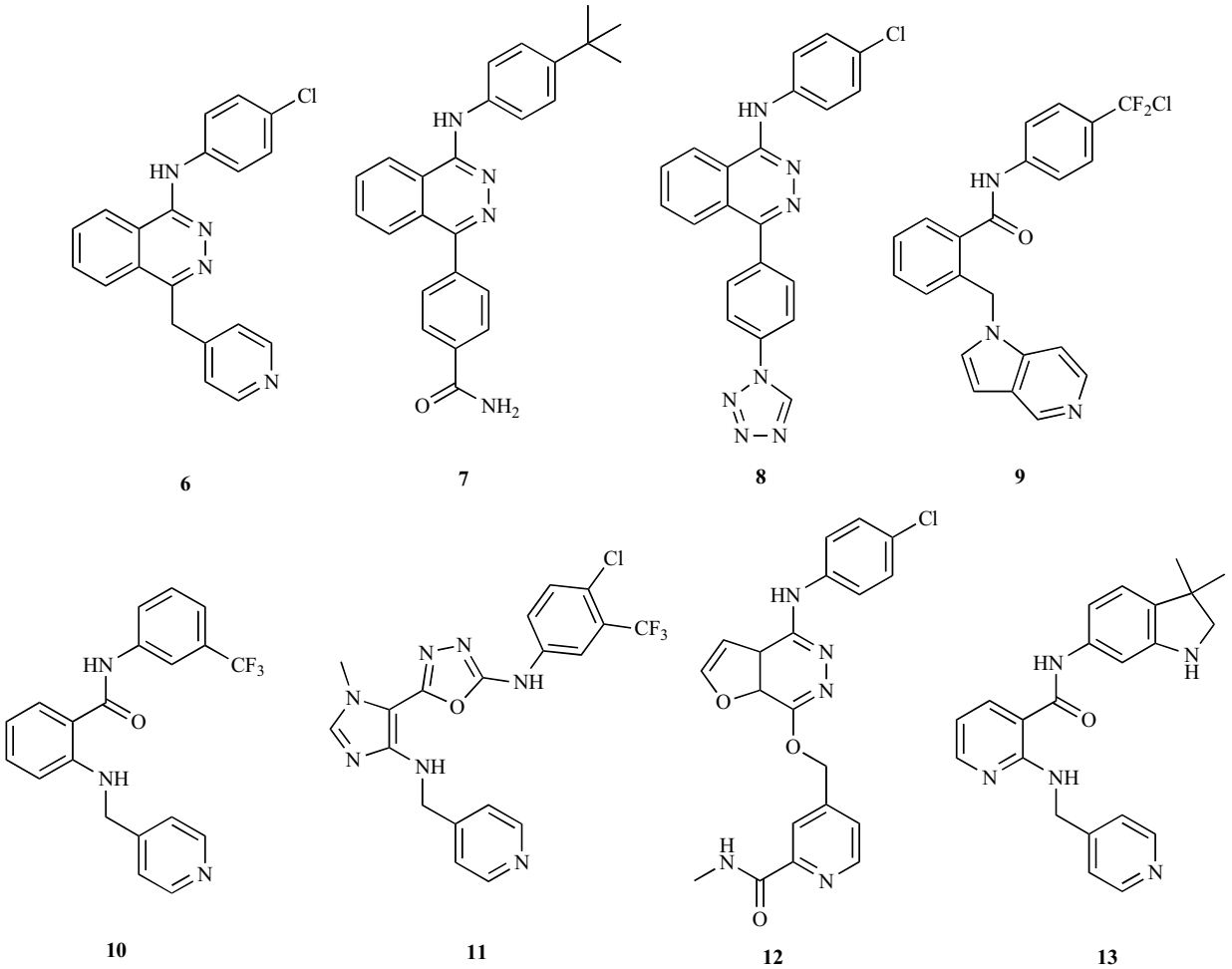


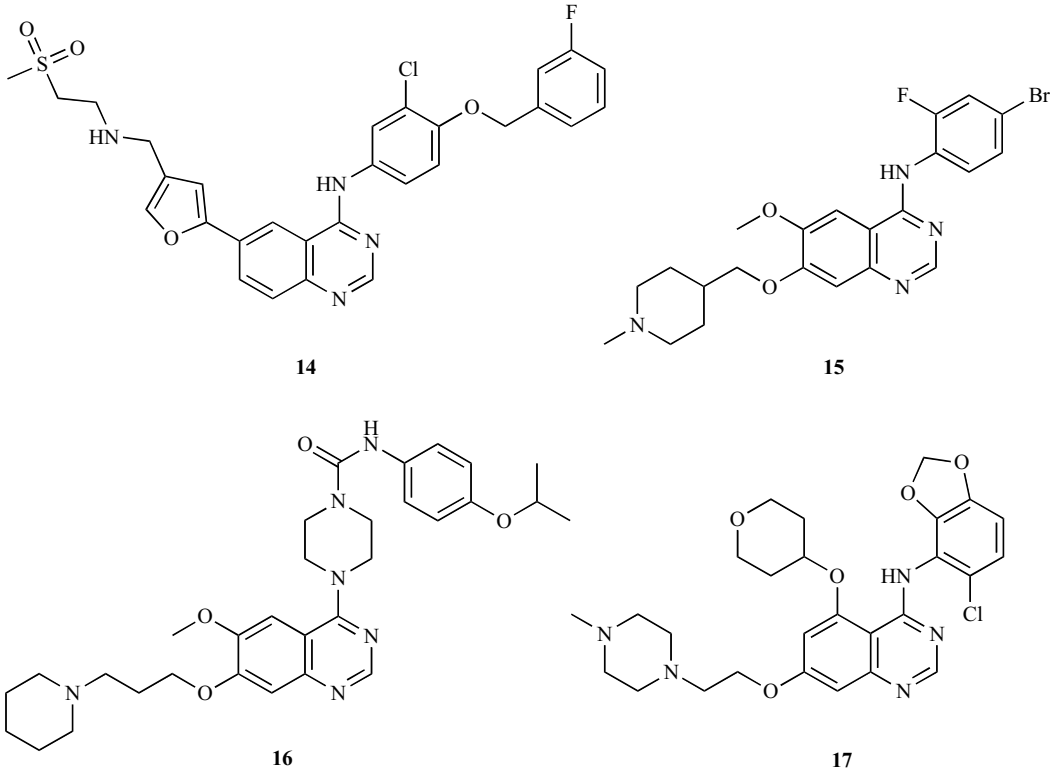
Fig. (3).

tionally, it was demonstrated that the pthalazine core can be replaced by an anthranilamide like **10**. In this case, the core structure is supposed to be conformationally constrained through an intramolecular hydrogen bond between the secondary amine and the carbonyl group, thus mimicking the pyridazine ring of the phtalazine core and keeping the two substituents in the right orientation [22]. The same effect is observed, if the pthalazine is replaced by an appropriately substituted nitrogen-containing five-membered ring, as for instance imidazole, pyrazole and isoxazole [23]. Even the incorporation of a second heterocycle between the core structure and the aniline moiety is possible, as was recently shown by the synthesis of the imidazolyloxadiazole **11** [24]. Based on the synthetic investigations described above, a general pharmacophore for the pthalazine-type multi-kinase inhibitors has been proposed. It includes: a.) A [6,6]fused aromatic system or a related cyclic system as core structure, b.) a *p*- or 3,4-disubstituted aniline group in position 1 of the phthalazine core and c.) a hydrogen bond donor attached to position 4 of the phthalazine through an appropriate linker (e. g. aryl or fused aryl).

It should also be mentioned that two compounds which are rather similar to Vatalanib recently have been published: In 2006, researchers at Bayer presented the furopyridazine Telatinib (BAY-579352) **12**, which is described as a potent inhibitor of VEGFR-2/3, PDGFR-β, and c-Kit. In a phase I clinical study with patients with advanced solid tumors, **12** Telatinib was found to be well tolerated [25], but up til now, no further data on its clinical efficacy have been published. Researchers at Amgen published Motesanib (AMG-706) **13**, a nicotineamide-based inhibitor. It is a potent and orally bioavailable inhibitor of VEGFR1-3, PDGFR, and c-Kit. It shows inhibition of angiogenesis *in vivo* and induces regression in a human epidermoid xenograft model in nude mice [26]. Currently, Motesanib is evaluated in three phase I studies with patients with advanced solid malignancies [27]. Recently, a close analogue of Motesanib was cocrystallized with VEGFR-2 and thereby, the binding mode of this class of compounds was elucidated [28]. This investigation revealed that the picolyl substituent of this Motesanib-like compound is located inside the ATP binding pocket and its nitrogen atom is engaged in a hydrogen bond to the hinge region, while both the nitrogen and the oxygen atom of the amide moiety form hydrogen bonds to residues of the so-called DFG motif at the far inner end of the binding pocket.

QUINAZOLINES – REVERSIBLE INHIBITORS

In the 1990s, the 4-anilinoquinazoline scaffold was exploited for the design and synthesis of highly selective kinase inhibitors. Theses efforts culminated in the developement of the EGFR-(epithelian growth factor receptor)-selective inhibitors Erlotinib and Gefitinib. Shortly afterwards, the 4-anilinoquinazoline structure proved to be useful for the design of dual- or even multiple-kinase inhibitors as well. This core was particularly used to create dual EGFR/HER2 inhibitors. The first representative of this class of compounds that is clinically used is Lapatinib **14** (Tykerb®, GW572016, GlaxoSmithKline). It was approved by the FDA in March 2007 for the treatment of advanced or metastatic breast cancer in patients whose tumors overexpress HER2 and who have received prior therapy with trastuzumab, taxanes, or anthracyclines [29]. In this therapy, it is combined with capecitabine, a prodrug of 5-fluorouracil. As Lapatinib is capable of crossing the blood-brain barrier, it is claimed to be useful for the treatment of brain metastases [30,31]. Another inten-



14 **15**

16 **17**

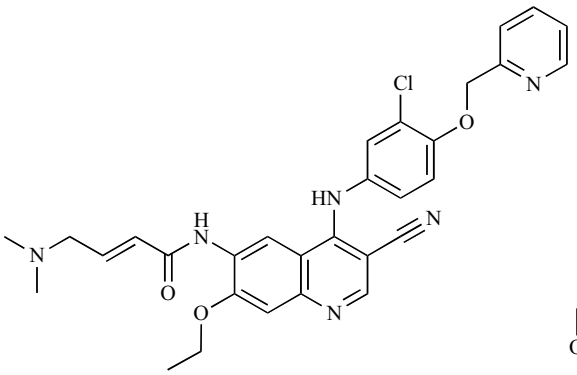
Fig. (4).

other intensively studied 4-anilinoquinazoline, Vandetanib **15** (Zactima®, ZD-6474, AstraZeneca) inhibits EGFR and VEGFR-1 in the nanomolar range, with additional activity against the receptor tyrosine kinases Abl (Abelson kinase) and RET (the product of the oncogen RET, which stands for "rearranged during transfection") [32]. Vandetanib was shown to cause significant tumor growth inhibition in a range of histologically diverse human xenograft models and is able to induce regression of established prostate cancer tumors [33]. It has also been shown to effectively reduce the growth of Gefitinib-resistant tumors in human xenografts, and might therefore be applicable to the treatment of tumors which have become resistant to EGFR-specific kinase inhibitors [34]. Moreover, Vandetanib has demonstrated antimetastatic effects in a human pancreatic tumor model, resulting in a marked reduction of lymph node and liver metastases [35]. In a clinical phase I study with Vandetanib with patients suffering from solid malignancies, daily oral doses of Vandetanib (<300 mg) were well tolerated, but had a poor efficacy, leading only to a disease stabilization in some patients [36]. In contrast to this, in another phase I study, four out of nine patiens with NSCLC showed an objective response [37]. Phase II trials with Vandetanib in patients with different tumor types, both as monotherapy and in combination with other antineoplastics are currently under way [38].

MLN518 **16** (CT-53518, Tandutinib) [39] is a quinazoline-based inhibitor of PDGFR-β (0.2 μM), Flt-3 (0.22 μM), c-Kit (0.17 μM), and CSF-1-R (3.43 μM) [40]. In contrast to the widely used c-Kit inhibitor Imatinib, MLN518 has proven to effectively inhibit Imatinib-resistant mutant isoforms of Kit. In a clinical phase II study with AML (acute myeloid leukemia) patients, two out of fifteen examinable patients showed a stable disease for more than 50 days, six patients acheived a reduction of peripheral blood and bone marrow blasts, but no partial or complete responses were seen. MLN518 elicited a low toxicity in AML cases which could make MLN518 an option for replacing Imatinib in the treament of these malignancies. Currently, MLN518 undergoes clinical evaluation for the treatment of refractory AML, metastatic kidney cancer, prostate cancer, and progressive glioblastoma [41]. Recently, researchers of AstraZeneca have published AZD0530 **17**, a 4-anilinoquinazoline that is a highly specific dual inhibitor of the tyrosine kinases c-Src (Rous sarcoma virus kinase) and Abl. AZD0530 inhibits both c-Src and Abl at low nanomolar concentrations, but it is highly selective over a broad panel of other kinases. Furthermore, AZD0530 was demonstrated to potently inhibit tumor growth in a c-Src-transfected fibroblast xenograft model and to significantly increase survival time in a highly aggressive, orthotopic model of human pancreatic cancer [42]. AZD0530 was also shown to induce apoptosis in 11 different NSCLC cell lines, including a cell line insensitive to Gefitinib, and to reduce tumor metastasis of human colon cancer cells in mice [43].

QUINAZOLINES – IRREVERSIBLE INHIBITORS

A number of attempts have been undertaken to create selective and irreversible inhibitors of EGFR by attaching an acroyl moiety to the 6-position of the quinazoline core. On binding to the catalytic site of EGFR, this moiety is brought into vicinity of the side chain of Cys773, a cystein residue located at the solvent-exposed periphery of the ATP-binding pocket which is unique to the erb family. The thiol group of this cystein then adds to the double bond of the acroyl residue, thus fixing the inhibitor covalently within the catalytic site. This concept has also been applied to the design of dual EGFR/HER2 or pan-Erb inhibitors. The most intensively studied one among these dual inhibitors is CI-1033 **18**



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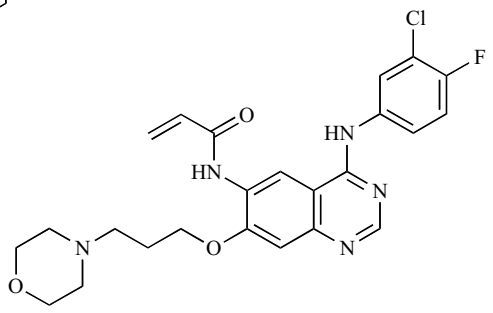

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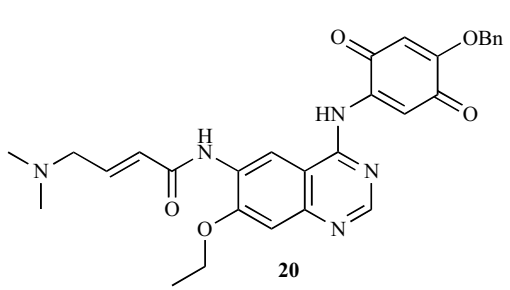

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Fig. (5).

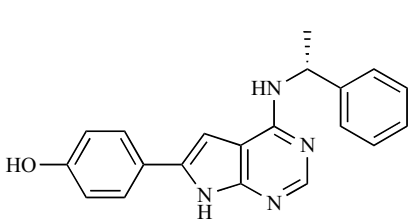
(PD183805, Canertinib, Pfizer) which irreversibly inhibits all four members of the erbB receptor tyrosine kinase family without affecting other receptor tyrosine kinases. When administered to athymic nude mice bearing xenografts of human A431 epidermoid carcinoma, NSCLC, oesophageal cancer and glioblastoma, it causes significant suppression of tumor growth [44,45]. Additionally, the concomitant administration of CI-1033 was found to have an enhancing effect on the therapy with ionizing radiation, leading to a prolonged inhibition of tumor growth in nude mice bearing colorectal cancer xenografts [46]. It was also demonstrated that CI-1033 enhances the cytotoxicity of the topoisomerase I inhibitor 7-ethyl-10-hydroxycamptothecin, the active metabolite of irinotecan, in a number of different cancer cell lines. This effect was accredited to CI-1033´s ability of inhibiting the breast cancer resistance protein (BCRP), an ATP binding cassette transporter which is involved in the phenomenon of multidrug resistance [47]. Furthermore, CI-1033 was found to induce caspase-independent apoptosis in a colorectal cancer cell line [48]. CI-1033 has been evaluated in a large number of phase I clinical studies both as monotherapy and in combination with other cytostatic agents, like docetaxel [49], or paclitaxel and carboplatin [50]. In 2005, data from a phase II trial of a CI-1033 monotherapy in patients with platinum-refractory or recurrent ovarian cancer were published. In this trial, a stabilization of the disease was achieved in a number of patients, but no responses were observed [51].

Crystallographic analyses of 4-anilinoquinazolines bound to the ATP binding site of EGFR have revealed that the nitrogen in position 3 forms an indirect, water mediated hydrogen bond to a conserved threonine residue of the hinge region. This water molecule can be replaced if a nitrile is introduced into the quinazoline core structure, thus leading to 3-cyanoquinolines which have been established as alternative lead structures of selective EGFR inhibitors [52]. 3-Cyanoquinolines have also been used for the development of dual EGFR/HER2 inhibitors. Based on this knowledge, a number of 3-cyanoquinolines bearing an acroyl substituent in position 6 have been synthesized by researchers at Wyeth as irreversible dual inhibitors of EGFR/HER2, leading to the clinical candidate HKI-272 **19** [53]. *In vivo*, HKI-272 reduces tumor growth, in HER-2- and EGFR-dependent tumor xeno-graft models. A phase I clinical trial with HKI-272 in patients with solid tumors is currently conducted. Preliminary published data analyses indicate that HKI-272 can stabilize the disease for over 6 month in some patients with NSCLC that has progressed after treatment with Gefitinib or Erlotinib. A phase II trial of HKI-272 in NSCLC patients has recently been initiated [54]. As far as can been deduced from the available data, HKI-272 might offer benefits to NSCLC patients who no longer respond to Erlotinib [55]. Recently, a similar strategy was applied to synthesize quinazolines like **20** as dual and irreversible inhibitors of EGFR and VEGFR. These compounds bear an acroyl moiety in the 6-position, that can react with Cys773 of the EGFR-active site. Additionally, they possess an aminobenzoquinone ring instead of the common aniline substituent. The benzoquinone moiety can react with Cys1045, a cysteine residue located inside the hydrophobic pocket of the VEGFR-2 active site which is usually occupied by the aniline ring of 4-anilinoquinazoline inhibitors, making this compound an irreversible inhibitor for both enzymes [56].

OTHER FUSED PYRIMIDINES AND RELATED COMPOUNDS

Besides the quinazoline core structure, a number of other fused pyrimidines has been evaluated as dual or multi-kinase inhibitors. The most intensively studied fused pyrimidines are two pyrrolo[2,3-*b*]pyrimidines, PKI166 **21** (CGP-75166, Novartis) and AEE788 **22** (NVP-AEE788, Novartis).

The first one, PKI166 originally was designed as a dual inhibitor of EGFR and HER2, but it appears to be more promiscuous than the quinazolines derivatives mentioned above, since it also exhibits activity against VEGFR-2 with an IC_{50}-value of 0.3 μM. PKI166 - alone as well as in combination with established antineoplastics - showed inhibition of tumor growth and metastasis in various nude mouse tumor models, including squamous cell carcinoma [57], renal cell carcinoma [58], pancreatic carcinoma [59], prostate cancer [60-62], and tongue cancer [63]. Interestingly, PKI166 was not only found to inhibit tumor growth and promote apoptosis of tumor cells, it also inhibited the formation of microvessels within the tumor. This effect was ascribed to its ability to lower EGFR-stimulated production of pro-angiogenic molecules like FGF (fibroblast growth factor) and VEGF, but direct inhibition of VEGFR might also contribute to this effect. Furthermore, PKI166 was shown to enhance the antitumor activity of the pan-VEGFR-inhibitor Vatalanib, indicating that a concomitant inhibition of angiogenesis and EGFR might be a feasible strategy for cancer therapy [64]. Although a phase I study with patients with advanced solid tumors proved PKI166 to be well tolerated [65], its clinical development was held due to hepatotoxic effects and instead of PKI166, its ethylpiperazine-substituted analogue AEE-788 was further investigated. In contrast to PKI166, AEE-



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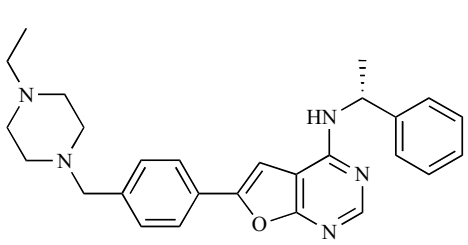

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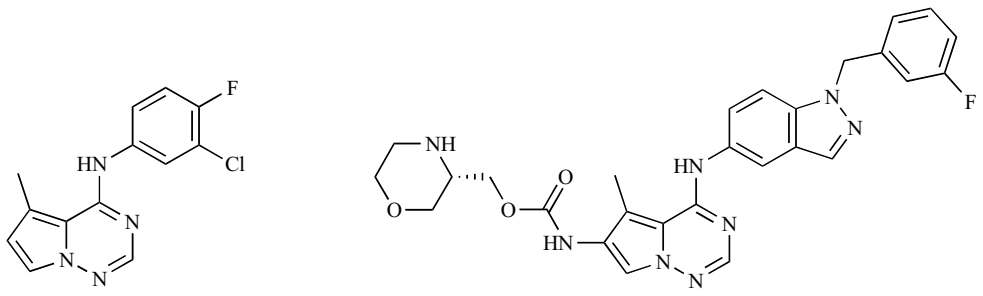
Fig. (6).



23 **24**

Fig. (7).

788 is a truly multi-kinase inhibitor which inhibits EGFR/HER2 and VEGFR-2 with nearly the same potency. Additionally, it possesses activity against Flt-3 [66]. AEE-788 was shown to inhibit tumor growth in several nude mouse tumor models, including head and neck [67,68], colon [69], thyroid [70-73], ovary [74], and pancreas cancer [75], as well as salivary adenoid cystic carcinoma [76]. AEE-788 was also found to reduce tumor incidence, tumor weight, and lymph node metastasis in a multidrug-resistant prostate cancer mouse model, making it an interesting option for the treatment of multidrug-resistant tumors [77]. In a recently conducted study, AEE-788 was shown to inhibit growth of several cholangiocarcinoma cell lines more potently than the EGFR-selective inhibitors Erlotinib and Gefitinib [78]. Currently, AEE-788 is evaluated in three clinical studies [79].

As a further variation of the inhibitors with quinazoline core, a number of pyrrolo[1,2-*f*][1,2,4]triazines were established as dual inhibitors of EGFR and HER2 by researchers at Bristol-Myers Squibb, starting with the moderately potent EGFR-inhibitor **23** (IC50 = 0.1 μM) as lead structure [80]. Enlargement of the aromatic substituent in the 4-position and the attachement of a second distal ring in this position improved both potency and selectivity of the lead compound for both EGFR and HER2, while position 5 and 6, which point towards the solvent-exposed region of the ATP binding site, were used to introduce various polar groups in oder to modify molecular properties like solubility or pharmacokinetic properties of the compounds [81,82]. These efforts finally lead to BMS-599626 **24**, a highly potent and selective dual EGFR/HER2 inhibitor (IC_{50}-values: 22 and 32 nm, respectively), which was selected to be evaluated preclinically. BMS-599626 inhibits the formation of EGFR/HER2 heterodimers and the growth of mouse salivary gland tumor cells *in vitro* as well as in an tumor allograft model *in vivo*. In the course of these studies, it was revealed that BMS-599626 inhibits EGFR in an ATP-competitive manner, but shows ATP-noncompetitive kinetics in the inhibition of HER2. This indicates that BMS-599626 adresses a different binding site on HER2 apart from the ATP-binding pocket, although its ATP-binding site is very similar to that of EGFR [83]. This might differentiate BMS-599626 from all other, purely ATP-competitive kinase inhibitors currently used and might be of interest in terms of treating kinase inhibitor-resistant tumor cells. Therefore, clinical studies with BMS-599626 for the treatment of solid malignancies are currently under way [84]. As a further modification of the quinazoline core structure, a series of anilinoalkenylpyrimidines **25** as dual EGFR/HER2 inhibitors were recently published.

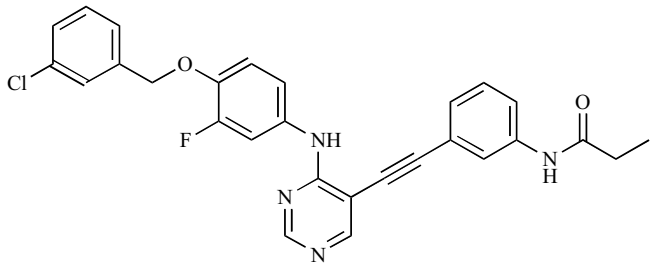


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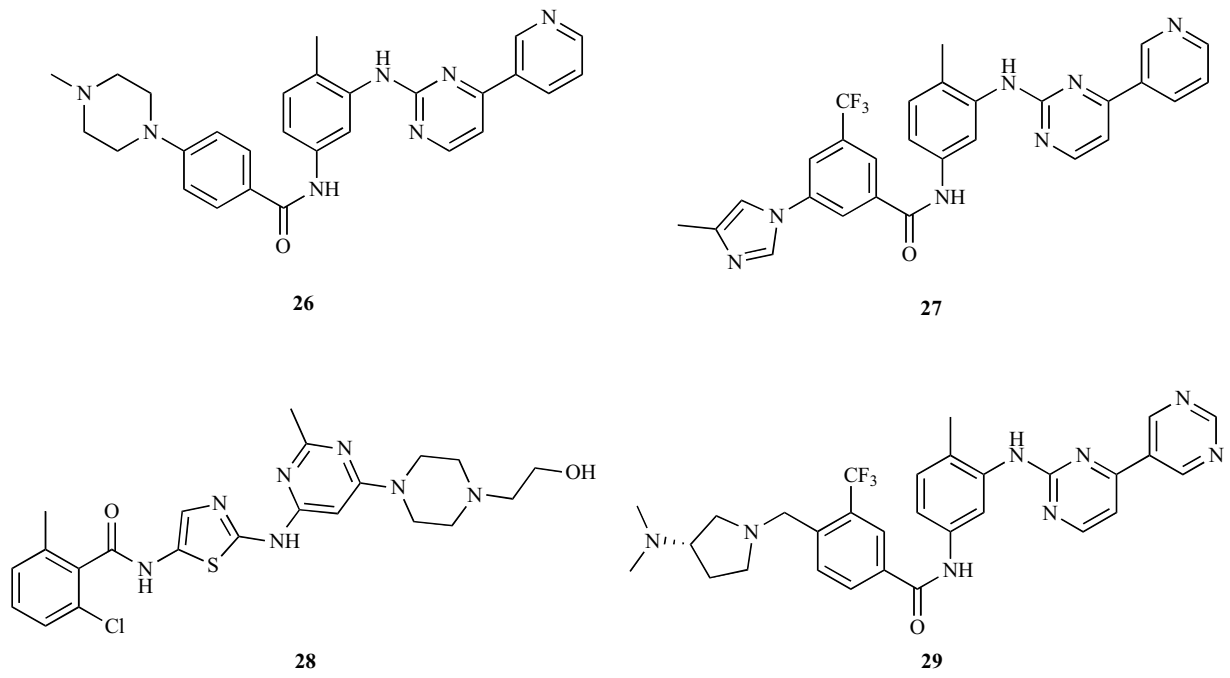
Fig. (8).

Since these "truncated quinazolines" possess activity against EGFR amd HER2 in the low nanomolar range, this example suggests that a potent ATP-analogue kinase inhibitor does not necessarily have to possess a fused heterocyclic core [85].

BENZAMIDES AND OTHER IMATINIB-LIKE COMPOUNDS

The benzamide Imatinib **26** (Gleevec®, STI-571, Novartis) was initially developed as a selective inhibitor of the tyrosine kinase Bcr-Abl, a fusion protein which is associated with the Philadelphia-chromosome-positive form of chronic myelogenous leukemia (CML), and is currently the standard therapy for this disease [86]. Later, it was shown that it also inhibits c-Kit and the PDGFRs [87]. Therefore, Imatinib was also established as a therapeutic option for the treatment of GIST. In contrast to most of the other multi-kinase inhibitors, Imatinib does not only occupy the ATP-binding site, but stretches out into the rear hydrophobic pocket of the catalytic cleft, making hydrophobic interactions with the amino acid residues of this pocket as well as hydrogen bonds with residues of the activation loop of the kinase.

Under therapy with Imatinib, a rapid development of resistance is frequently observed. As this phenomenon is due to the appearance of insensitive mutant c-Kit isoforms, researcher try to create Imatinib analogues which also inhibit these variants of c-Kit. One of these derivatives, Dasatinib **27** (Sprycel®, BMS-354825, Bristol-Myers Squibb), was found not only to inhibit wild-type c-Kit, but also three



26 **27**

28 **29**

Fig. (9).

Imatinib-resistant c-Kit activation loop mutants (D816Y, D816F, and D816V). Based on these findings, Dasatinib is currently evaluated as a therapeutic option for the treatment of Imatinib-resistant GIST [88]. Data from a first clinical phase I study with patients suffering from treatment-resistant GIST or other refractive solid tumors indicate that Dasatinib can be savely administered [89], and further studies will be required to evalute the use of Dasatinib as an alternative treatment of GIST. Besides of Dasatinib, two other analogues of Imatinib, Nilotinib **28** (Tasigna®, AMN107, Novartis) [90], and INNO-406 **29** (NS-187, Innovive) [91], are currently evaluated for the treatment of GIST. INNO-406 was discovered as an inhibitor of Bcr-Abl [92], but it also proved to inhibit wild-type c-Kit, although with a lower potency than Imatinib [93]. The treatment of Imatinib-resistant GIST with INNO-406 is currently evaluated in clinical phase I studies.

DIPHENYLUREAS

Another structure that is frequently used for the design of multi-kinase inhibitors is the diphenylurea motif. The first approved compound of this class of agents is the orally active multi-kinase inhibitor Sorafenib **30** (Nexavar®, BAY43-9006, Bayer-Schering) [94]. This compound was developed as an inhibitor of Raf-1, a kinase that is part of the MAPK-(mitogen activated protein kinase)-signalling pathway, through classical medicinal chemistry out of a high-throughput-hit. Further evaluation revealed that Sorafenib also inhibits several other tyrosine kinases, including VEGFR-1-3, Flt-3, c-Kit, PDGFR-β. That means, Sorafenib does not only target the MAPK-signalling pathway, but also kinases which are involved in angiogenic processes. The binding mode of Sorafenib to Raf-1 was elucidated by crystal structure analysis: While the pyridine part of the molecule is located within the ATP binding pocket, making two hydrogen bonds to the peptide backbone of the hinge region, the urea moiety forms two hydrogen bonds to the DFG motif, one with the backbone of the aspartate included in the DFG motif, the other one with the side chain carboxylate of a glutamic acid residue, and the trifluoromethylphenyl ring occupies a hydrophobic cavity located at the far inner end ot the catalytic site. This binding mode is very similar to that observed with Imatinib.

In December 2005, the FDA approved Sorafenib for the treatment of metastatic renal cell carcinoma. Due to its advantageous safety profile, Sorafenib is currently evaluated for the treatment of other types of cancer: In a recently conducted phase III study with patients suffering from advanced, refractive clear cell renal cell carcinoma, Sorafenib proved to prolong the median progression-free survival time [95]. In another clinical phase II study, the use of Sorafenib for the treatment of hepatocellular carcinoma was investigated, but in this case, only moderate results with respect to the reduction of tumor progression could be observed. The authors who reported these data hypothesize that in this case, a combination of Sorafenib with a classical cytostatic drug might lead to better results [96]. Possible uses of Sorafenib in combination with other established antineoplastic agents are also currently under investigation, as for instance combinations with doxorubicin [97] and oxaliplatin [98].

Based on the same structural concept, several companies recently reported the synthesis of various novel multi-kinase inhibitors, all of which consist of a bicyclic core structure, which occupies the ATP binding site and a diphenylurea motif which binds to the DFG motif. Among these compounds are thienopyrimidine ureas **31**, which show potent inhibition of several kinases of the VEGFR and PDGFR families (VEGFR-1-3, Flt-3, c-Kit, CSF-1-R, and Tie-2) with IC_{50}-values in the nanomolar range [99,100]. Another type of diphenylurea compounds which shows activity against VEGFR and Tie-2 was prepared on the basis of a 4-amino-

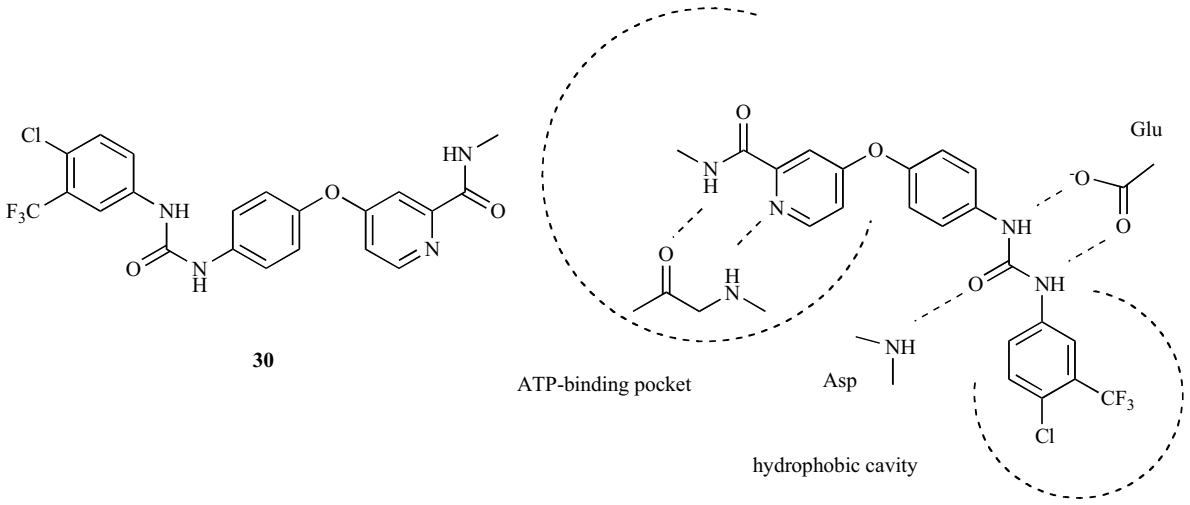


Fig. (10).

furopyrimidine scaffold **32** [101,102]. Recently, researchers at GlaxoSmithKline published a series of novel benzimidazoles like **33**, which act as dual inhibitors of VEGFR-2 and TIE-2 [103]. These compounds possess a carbamate functionality, which points towards the solvent exposed region of the ATP binding pocket. This carbamate can be replaced by other similar moieties, like carbonic acid and sulfonamides, thus giving the opportunity to attach a variety of different groups in order to influence physicochemical and pharmacokinetic properties of the inhibitor. Other bicyclic core structures that have been used in connection with the diphenylurea motif are 3-aminoindazoles **34** [104], as well as substituted benzimidazoles and -oxazoles [105].

THIENOPYRAZOLES

In the recent past, researchers from Abbott introduced 1,4-dihydroindeno[1,2-*c*]pyrazoles **35** as another interesting class of antiangiogenic multi-(tyrosine)-kinase inhibitors. After having identified **35** as a moderately potent inhibitor of VEGFR (IC_{50}=1.2 μM) *via* high-throughput-screening, its binding mode to VEGFR and orientation within the ATP binding pocket was analyzed by docking studies, which indicate that both nitrogens are engaged in hydrogen bonds to the hinge region, while the thiophene ring is deeply buried inside the hydrophobic back of the ATP binding pocket and the phenyl ring points towards the solvent exposed region of the catalytic site. The potency of this lead compound was improved by adding various polar heterocycles to the solvent exposed end of the molecule, with **36** being the most potent derivative. **36** showed potent inhibiton of VEGFR-1, -2, -3, and c-Kit in the nanomolar range and was found to be active *in vivo* after oral administration [106]. During optimization of the lead compound, the authors also tried to improve both selectivity and potency of their compound by attaching a DFG motif-binding phenylurea moiety to the thiophene ring, which lead to highly potent compounds like **37** [107,108]. Unfortunately, ADMET-(absorption, distribution, metabolism, excretion, and toxicity)-investigations of the phenylurea-substituted compounds like **37** revealed unfavorable pharmakokinetic properties with short half-lives and low

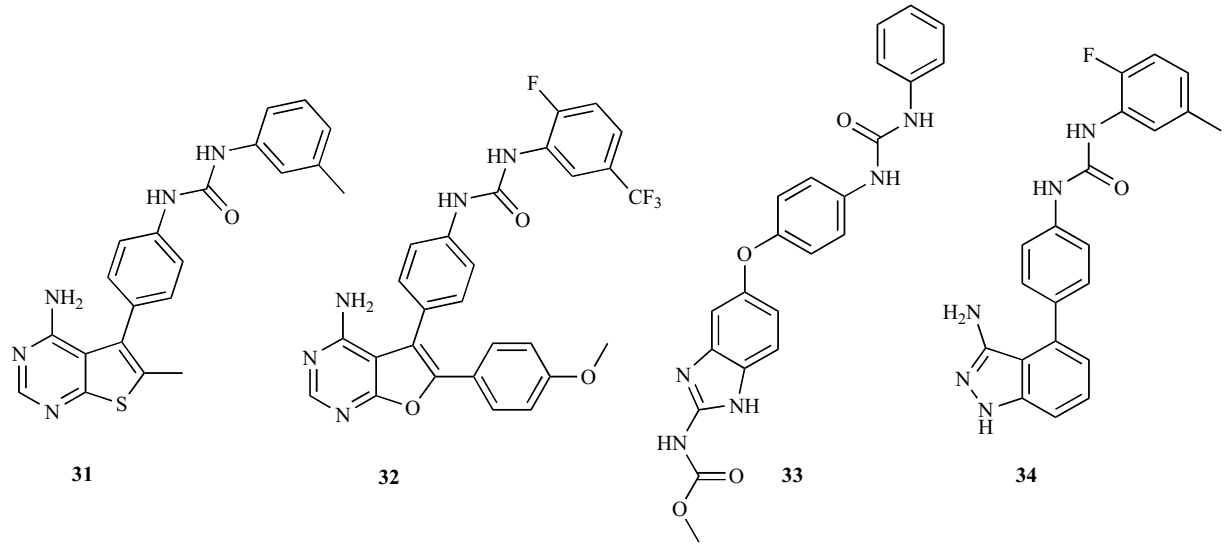


Fig. (11).

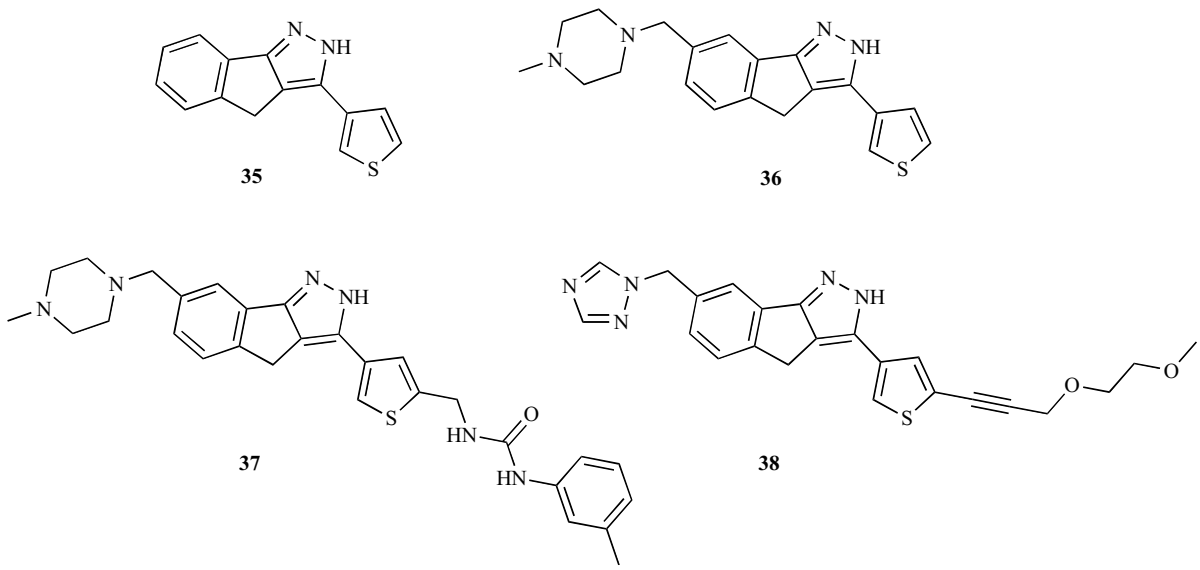


Fig. (12).

bioavailabilities. As an alternative to the urea-substituted compounds, derivatives substituted with a phenylpropargyl ether like **38** were discovered, which displayed an oral activity in the estradiol-induced murine uterine edema model (ED_{50} = 3 mg/kg) superior to Sunitinib (ED_{50} = 9 mg/kg). However, it also showed a high affinity to the hERG potassium channel. However, it was possible to reduce the hERG-affinity with the aid of docking studies on the channel protein [109].

DISCUSSION AND CONCLUSIONS

The first multi-kinase targeted anticancer drugs, which are already therapeutically used have shown very convincing results in the treatment of various tumor types, while other, also very promising drug candidates achieved only limited efficacy, or only were effective in certain cancer types. Maybe, in spite of their often low degree of specificity, some novel multi-kinase inhibitors will only reach maximum efficacy in tumors with defined kinase overexpression profiles that closely correspond to the inhibition preferences of the inhibitor, rather than being "magic bullets" for the treatment of a broad range of cancer types. This implicates that maybe, only a defined subgroup of all patients who suffer from a certain type of cancer could benefit from the administration of a multi-kinase inhibitor, while others would only experience the adverse effects which are associated with the therapy. In order to gain the ability to predict the therapeutic benefit that can be expected for an individual patient, it will be necessary to further characterize the pharmacological profile of the different multi-kinase inhibitors by testing them against a really broad panel of protein kinases and additionally by searching for other, non-kinase targets that might be relevant for their therapeutic potency and their safety. Recently, such an investigation was performed with a limited number of approved kinase inhibitors and clinical drug candidates using a panel of 317 different human protein kinases [110]. This work proved that all kinase inhibitors tested – including even the "selective" EGFR-inhibitors Erlotinib and Gefitinib - address a more or less large number of other kinases apart from their known targets, and revealed remarkably different selectivity profiles for even very closely related inhibitors like Imatinib and Dasatinib. More investigations like this are required to provide a deeper insight into the actual impact each inhibitor exerts on the whole kinome. Once more profound knowledge about the multi-kinase inhibitors and their targets has been acquired, thorough design of further clinical trials including target-based patient selection might lead to more specific information on the possibilities each kinase inhibitor offers for the treatment of a specific type of cancer and the individual clinical outcome that has to be expected.

Apart from this, a truly objective assessment of the value and the safety of newly developed multi-kinase inhibitors within a review article is generally hampered by a phenomenon that is usually called the "publication bias". This means that plenty of information is published about successful drug candidates, whereas information on unsuccessful compounds is only scarcely available. Therefore, someone who reviews the currently published literature can easily get the impression that the majority of the currently developed multi-kinase inhibitors exhibits an excellent therapeutic perspective, but this does not necessarily have to reflect the actual overall situation.

Since the majority of the clinical trials conducted with the drug candidates presented in this review demonstrates that these agents are comparatively well tolerated, some multi-kinase inhibitors might be safely combined with other anticancer drugs. Such combination therapies might enhance the clinical efficacy of the therapy without aggravating the toxic side effects.

However, when talking about the unwanted side effects of multi-kinase inhibitors, certain facts have to be kept in mind: First, the multi-kinase are still relatively new on the market and only limited clinical experience has been collected during their use. That means that some only rarely occurring but nevertheless severe side effects of these agents might still wait for their discovery [111]. For instance, the

occurrence of acute heart failure under the treatment with Sunitinib **1** has been outlined in a number of recent publications [112], as well as severe skin irritations, which were observed during the therapy with Sunitinib and Sorafenib [113]. In both cases, it remains unclear, whether these side effects are due to the kinase-inhibitory properties of Sunitinib and Sorafenib or are caused by pharmacological effects on other targets, which have not yet been revealed. The analysis of the origin and nature of these side effects and their eradication by structural changes of the molecule or optimization of dosage and pharmaceutical form will probably turn out to be rather complex, due to the complexity and the interdependency of the effects which are caused by a drug which simultaneously addresses several targets, especially, as long as not all of these targets are known.

Second, the multi-kinase inhibitors, which have been developed so far are used for cancer therapy, where generally more severe side effects are accepted than would be for any other medication, which, for instance, is used to fight an enduring, but not life-threatening disease. Furthermore, the side effects of these novel anticancer drugs are always compared to the often very grave side effects of classical antitumor drugs, which can also make them appear to be milder than they objectively are. These facts and examples clearly illustrate that much more information obtained from a large number of patients has to be collected in order to provide a more informative picture of the properties and the true therapeutic value of the novel multi-kinase inhibitors. When more in-depth knowledge about successful multi-kinase inhibitors and their complex structure-activity-relationships is acquired, researchers might proceed to the rational design of "tailor-made" multi-kinase inhibitors for certain purposes instead of discovering them by chance during their search for selective inhibitors. In conclusion, multi-kinase inhibitors may offer a promising alternative to classical anticancer drugs, but the scope and limitations of their application still have to be explored.

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