Recent Progress of Src Family Kinase Inhibitors as Anticancer Agents

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Abstract: Src family of protein tyrosine kinases (SFKs) play key roles in regulating signal transduction in cellular processes. However, hyper-activated SFKs lead to uncontrolled cell proliferation and cancers. Many SFKs inhibitors were designed and synthesized as anticancer agents in the past several years and great progress has been made. Herein, some predominant examples of SFKs inhibitors recently developed are reviewed and special attentions are paid to the most important ATP binding site inhibitors.

Key Words: Protein tyrosine kinases, Src family kinases, kinase domain, inhibitor, anticancer, drug design, SH2, SH3.

1. INTRODUCTION

It has been almost a century since Peyton Rous firstly discovered that sarcoma virus (RSV) could cause cancer [1] and more than 40 years since he received the Nobel Prize in Physiology or Medicine in 1966 for his important contribution to cancer research. That was the beginning of the Src research. Then during the decades of 1970s to 1990s, studies on RSV developed fast and yielded fruitful achievements: Src derives from the proto-oncogene *c*-src; Src is a non-receptor protein kinase and catalyze the transfer of γ-phosphoryl group from ATP to the hydroxyl group of specific tyrosine (Tyr) residues in proteins; Src activity is regulated by intermolecular interactions controlled by its own tyrosine phosphorylation [2-5].

In light of the crucial cellular signaling processes *c*-Src involved in, it is obviously that *c*-Src activation has been implicated in the development and progression of various human cancers [6, 7]. In order to cure cancer by retarding the signal transduction of abnormal cell proliferations, Src inhibitors were proposed and have made great progress in the recent years. Both structure-based drug design and screening-based lead compound identification approaches have significantly impacted the discovery of Src inhibitors. The scope of known Src inhibitors includes natural products, peptide mimetic compounds and *de novo* designed non-peptides, ATP-related analogues, and a plethora of small molecule inhibitors that were derived from screening of chemical libraries [8-10].

2. Src KINASES IN CANCER

The observation that *c*-Src kinase activity elevated in various human cancers has prompted many investigations that aim at elucidating the mechanism of this activation. The most obvious collection of data is available on colon cancer. Several investigations showed that the specific activity of the non-receptor protein tyrosine kinase *c*-Src is increased 5-8 folds compared to normal mucosa in premalignant lesions as well as in the majority of colorectal adenocarcinomas, and those differences in *c*-Src activity correlate to tumor progression [11, 12]. Similar to the results observed in colon cancer, 20-folds higher elevated Src kinase activity compared to normal tissues has been found in human mammary carcinoma cell lines [13-15]. In addition to colon cancer and breast cancer, elevated Src activity has also been reported in many other epithelial tumor entities, including pancreatic [16], lung [17], ovarian [18], esophageal [19] and gastric cancer [20] as well as melanoma [21] and neuroblastoma [22], further corroborating a role of these kinases in tumorigenesis. And some other researches supported that Src kinases were also in control of VEGF-driven angiogenesis [23] (Table **1**).

Although it is not clear how Src is activated during the tumor genesis or how Src takes part in the whole process**,** recent research results showed that Src plays some important roles in the following procedures [24-26]. Src is activated by numerous extracellular stimuli including the ligands binding to receptor protein tyrosine kinases (RPTKs), through contacting with the extracellular matrix, and by G-protein coupled receptors. When a ligand binds with RPTK, the receptor is autophosphorylated. And then the receptor kinase is activated and SH2-mediated docking sites are formed at the cytoplasmic surface of the RPTKs. This allows transient signaling complexes emerge and SFKs to be recruited and activated. Src can phosphorylate receptors directly, sometimes at the residues which are not normally phosphorylated during receptor autophosphorylation, creating recruitment sites for SH2-containing molecules. Provocatively, synergistic changes in the expression of Src and RPTKs have been correlated with tumorigenesis derived from the pancreas, breast and lung, and often correlate with poor clinical prognosis [3].

3. SFKs' STRUCTURES AND THE Src INHIBITION MODES

Usually the Src kinase structure consists of four domains: a unique region, which varies among the family members,

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Table 1. The Classification of SFKs and their Relationships with Cancer

SFKs	Expression Tissues	Notes
Blk	B cells	
Fgr	Myeloid cells, B cells	Over expressed in some leukemias and lymphomas
Fyn	Ubiquitous	T-cell-specific isoform (Fyn T)
Hck	Myeloid cells	Two different translational starts
Lck	T cells, NK cells, brain	Over expressed in T-cell acute lymphocytic leukemias
Lyn	Brain, B cells, myeloid cells	Two alternatively spliced forms
Src	Ubiquitous	Over expressed in mammary, colon and other cancers
Yes	Ubiquitous	Expressed in colon, malignant melanoma and other cancers
Yrk	Ubiquitous	

followed by the SH3, SH2, and tyrosine kinase domains (SH1) [27].

SH2 domains consist of about one hundred amino acids. In general, SH2 domains are compact globular modules with common structural properties, including a central antiparallel β-sheet core flanked on each side by a α-helix [28, 29]. The common-fold and phosphortyrosyl-binding properties of the SH2 domain belie its versatility as a protein–protein recognition module. It is believed [30] that the conserved residue Arg 175 in the SH2 domain is critical for phosphotyrosine recognition and Trp 260 at the extreme N-terminus of the kinase domain is important for autoinhibition. In the autoinhibited form of Src kinases, the SH2 domain binds the phosphorylated C-terminal tail, and the SH3 domain binds the linker segment between the SH2 and kinase domain, which forms a polyproline type II helix.

SH3 domains own about 60 amino acids and consist of two three-stranded antiparallel β-sheets packed against each other, at angles of about 90°, resulting in a β-barrel-like structure [31, 32]. On the surface of the SH3 domain, these residues interact with two binding sites of a recognition platform, consisting of highly conserved aromatic residues (Trp, Tyr or Phe) and a proline [33].

Src kinase domains share the main fold characteristic of all tyrosine kinases [34]. The N-terminal lobe is composed of five β-sheets and a single α-helix, termed the C helix, which is an important component of the regulatory mechanism deployed in Src kinases. The ATP binds within a deep cleft formed between the two lobes of the kinase domain. Though the ATP binding site is highly conserved**,** the structure in the regions around the ATP binding site does afford some key diversities for new drug design and have potential applications in drug discovery.

Inhibition of Src kinase activity presents a good choice to block uncontrolled cell growth and has potential therapeutic utilities in developing cancer treatments. Though no Src inhibitors have been launched to the market, the researches have achieved big progress and verified many active compounds as potent drug candidates. For example, bosutinib (SKI-606, **19**) from Wyeth, a dual inhibitor of Src and Abl kinases has currently been in phase II clinical trials [35, 36].

According to the different inhibition locations on the enzymes, Src inhibitors may be categorized into three major inhibition modes: Src kinase domain inhibitors, SH2 inhibitors and SH3 inhibitors. The kinase domain inhibition mode is the most important and potent strategy to inhibit Src activities, including using the binding-site inhibitors and some ATP analogs.

4. RECENT PROGRESS OF SFK KINASE DOMAIN INHIBITORS

Src kinase domain inhibitors can be defined as the compounds that could potentially inhibit the binding of ATP or the substrate proteins to the Src catalytic domain thus inhibit the function of Src kinase. They can be classified as ATP-binding site inhibitors and substrate binding site inhibitors roughly. Over the past few years, numerous reports have covered the design, synthesis and biological evaluation of SFK inhibitors. From the optimization programs of a variety of lead structures or natural products, several compounds have passed the hurdles of preclinical research and entered clinical development. Currently, there are at least four Src inhibitors in various phases of clinical evaluation (Table **2**).

4.1. Src Kinase Domain Inhibitors' Features

Initially, inhibition of PTKs by ATP-directed compounds was considered less advantageous than substrate based inhibitors due to the assumption that the catalytic domain among different protein kinase families were highly conserved and selectivity of ATP-competitive inhibitors would be very difficult to achieve.

But the fact that selective inhibitors of different PTKs were discovered one after another in the recent years erases the worry of selectivity. The progress made in the crystallization of protein kinases, especially in complexes with ATP-site binding inhibitors, proved that there would be small differences in the region that are not critical to ATP binding site. For example, two small regions that have been exploited in the protein kinases were the hinge region nearby the

Table 2. The Reported Src Inhibitors in Clinical Trials in Present

Src Inhibitors	Cancer Categories	Trial Phase	Company
SKI-606	Breast cancer	Phase II	Wyeth
AZD0530	Prostate cancer	Phase II	AstraZeneca
BMS-354825	CML	Phase III	Bristol-Myers-Squibb
SKS-927	Colon cancer	Preclinical research	Wyeth

purine district, and the kinase specificity pocket which was at the head of the hinge region.

In the past ten years, striking progress has been made in the Src kinase inhibitor field. A number of valid scaffolds have been used for Src kinase inhibitor design, including quinazolines, 3-quinolinecarbanitriles, pyrazolopyrimidines, indolinones, benzotriazine and some other compounds, as described in this paper.

Generally, the ATP-binding site inhibitors contain a largely main conjugation structure, which was a heteroaromatic ring system in most cases, involved in the hydrophobic interactions between the planes of their aromatic ring systems of the hinge region, and a hydrophobic substituent that extended into the kinase specificity pocket. Some water soluble substituted groups like *N*-methylpiperazine and ether chains that would direct into the water solution out of the kinase domain were also accepted, for the purpose of improving the water solubilities of these molecules.

4.2. Natural Compounds

Natural Src kinase inhibitors including flavonoids, erbstatin, lavendustin A, herbimycin A and staurosporines, etc. These compounds have been reviewed by Paul W. Groundwater [37] and Keykavous Parang [38]. In the recent years, some new natural compounds were discovered as the Src kinase inhibitors. Jin Woo Kim etc reported [39] that the natural compounds oximidines I (**1**) and II (**2**) (Fig. **1**) which were isolated from *Pseudomonas* inhibited the growth of 3Y1 rat fibroblasts cells transformed with *v*-src oncogenes at 27 ng/mL and 14 ng/mL, 15-30 folds lower concentrations than that of the parent 3Y1 cells.

In another HTS-supported lead-finding process, Carsten Puder etc found some terphenyl quinines [40] were remarkable inhibitors of Src kinase from the screening of more than 80,000 natural products of plants and microbial origins. The most active compound (**3**), terphenylquinone, inhibited Src kinase with an IC_{50} value of 3.9 μM. And the other two hits, compounds (**4**) (IC_{50} 14.5 μM) and (**5**) (IC_{50} 23.6 μM) also exhibited moderate Src inhibition activities.

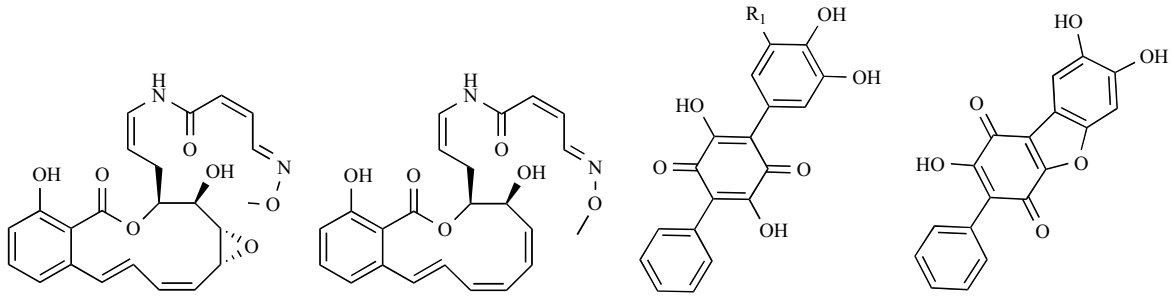
4.3. Anilinoquinazoline

The first synthesized [41] potent selective small molecule inhibitor of protein tyrosine kinase was PD-153035 (**6**) (Fig. **2**), which was an ATP competitive inhibitor of the epidermal growth factor receptor tyrosine kinase (EGFR), based on a quinazoline skeleton. From then on, a lot of 4-anilinoquinazoline derivatives were designed and prepared, and several of them were evaluated as potent protein tyrosine kinase inhibitors, such as Iressa (ZD-1839, **7**) and Tarceva (OSI-774, **8**).

Quinazoline skeleton also plays important roles as Src inhibitor template. The scientists from AstraZeneca reported several new series of quinazoline derivatives with excellent potency and selectivity in the recent years. The lead compound (**9**) had an IC_{50} of 37 nM towards Src kinase [42].

The 4-aniline groups were optimized by replacing different heteroaromatic amines at C-4 position. Benzofuran, indolin and benzodioxole derivatives were also brought in as the 4-position substituents. In each series, some potent compounds were discovered. For example, compounds (**10**-**13**) showed Src kinase inhibiting activities with IC_{50} 5-10 nM [42]. Among these compounds, the 4-aminobenzodioxole quinazoline (**13**) was identified as providing the optimum profile of selectivity, enzyme inhibition and cellular potency.

Further optimization was based on a 4-aminobenzodioxole quinazoline scaffold. A novel subseries of C-5-substituted



1 **2** **3: R_1 = OH; 4: R_1 = H** **5**

Fig. (1). Some natural compounds as Src kinase inhibitor.

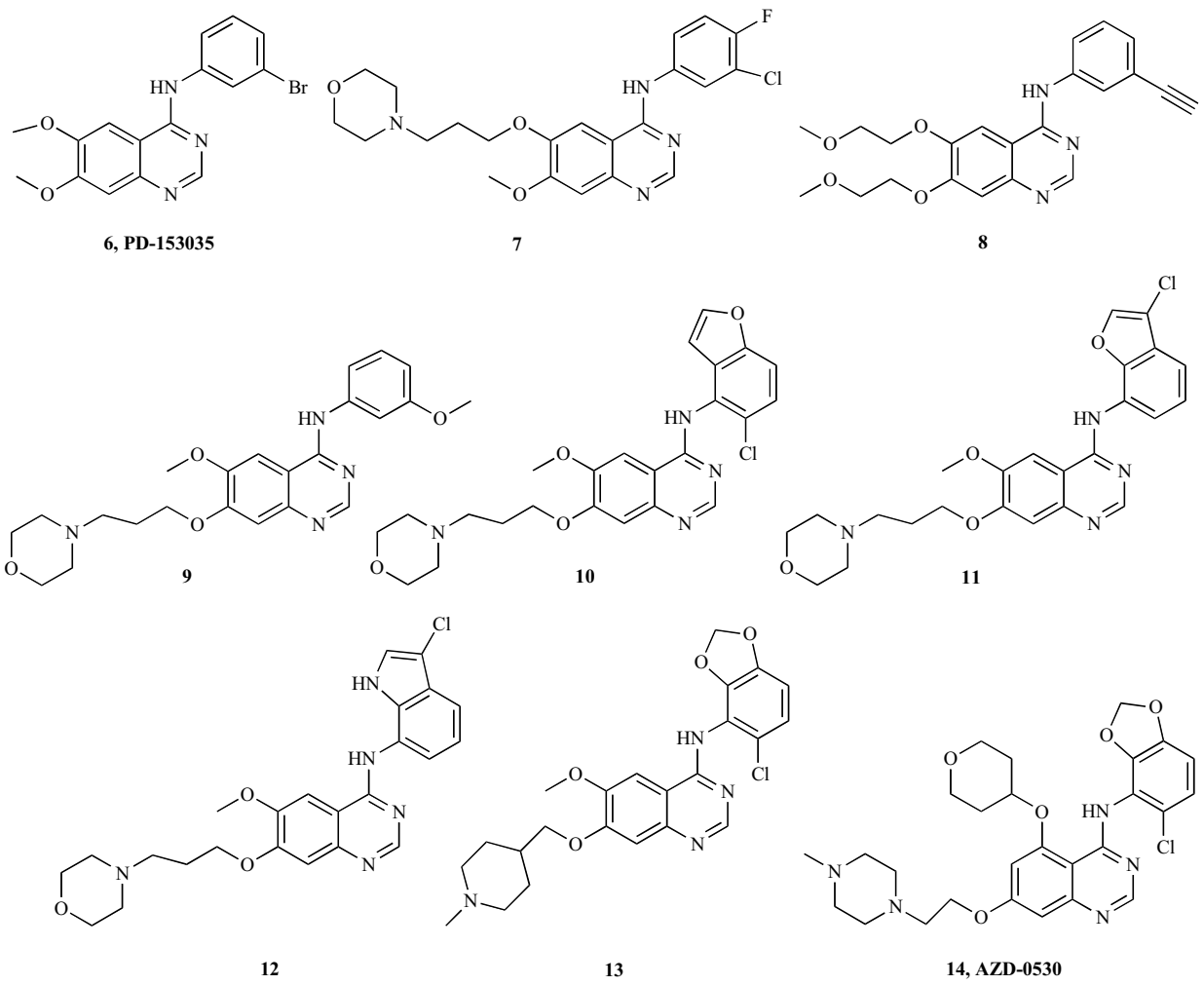


Fig. (2). The 4-anilinoquinazolines Src inhibitors and the clinical drug candidate AZD0530 (**14**).

quinazoline displayed high affinity and specificity for SFKs over a panel of recombinant protein kinases. AZD0530 (**14**) was the most potent one of these compounds; it inhibited *c*-Src and Abl enzymes at nanomolar concentrations and was highly selective over a range of other kinases. AZD0530 (**14**) also displayed excellent pharmacokinetic parameters in animal preclinical research, and has been in clinical trials for cancer treatment.

4.4. 3-Quinolinecarbanitriles

The 3-quinolinecarbonitrile core was the most important Src kinase inhibitor scaffold, which was designed based on the known quinazoline template. In 2000, a homology model of EGFR was constructed and a binding research of the model with PD-153035 (**6**) was carried out. This binding result suggested that the 3-nitrogen atom of the quinazoline ring would bind with the backbone of EGFR by two H-bonds through a H_2O molecular bridge [43]. It was assumed that the 3-nitrogen could be replaced by a carbon atom containing an electron-withdrawing group, such as a nitrile group. Several 3-substituted quinoline analogs of PD-153035 (**6**) were prepared, and the first successful 3-quinolinecarbanitrile agent, 4-(3-bromoanilino) -6, 7-dimethoxy-3-quinoline -carbonitrile (**15**), was obtained as a potent and selective EGFR kinase inhibitor [43].

Subsequent reports showed that variation of the substituents on the 4-anilino group of the 3-quinolinecarbonitrile core changed the kinase specificity from EGFR to either Src [44, 45] or MAPKK [46, 47].

In the past several years, scientists from Wyeth have achieved big progress in discovering new 3-quinolinecarbanitrile Src kinase inhibitors, and there have been several drug candidates, such as SKI-606 (**19**) and SKS-927 (**25**), being in the oncology clinical trials.

The first big improvement was started with compound (**15**), a Src inhibitor with 4-(3-bromo) aniline substituting group. After screening some analogues of 4-halogenated aniline-3-quinolinecarbanitriles, the best substituted group 2, 4-dichloro-5-methoxy phenyl was discovered (**16**). In order to improve the soluble properties of these compounds, some water-soluble groups, such as some ether chains, piperidyl group, morpholino group *et al.*, were introduced to the struc-

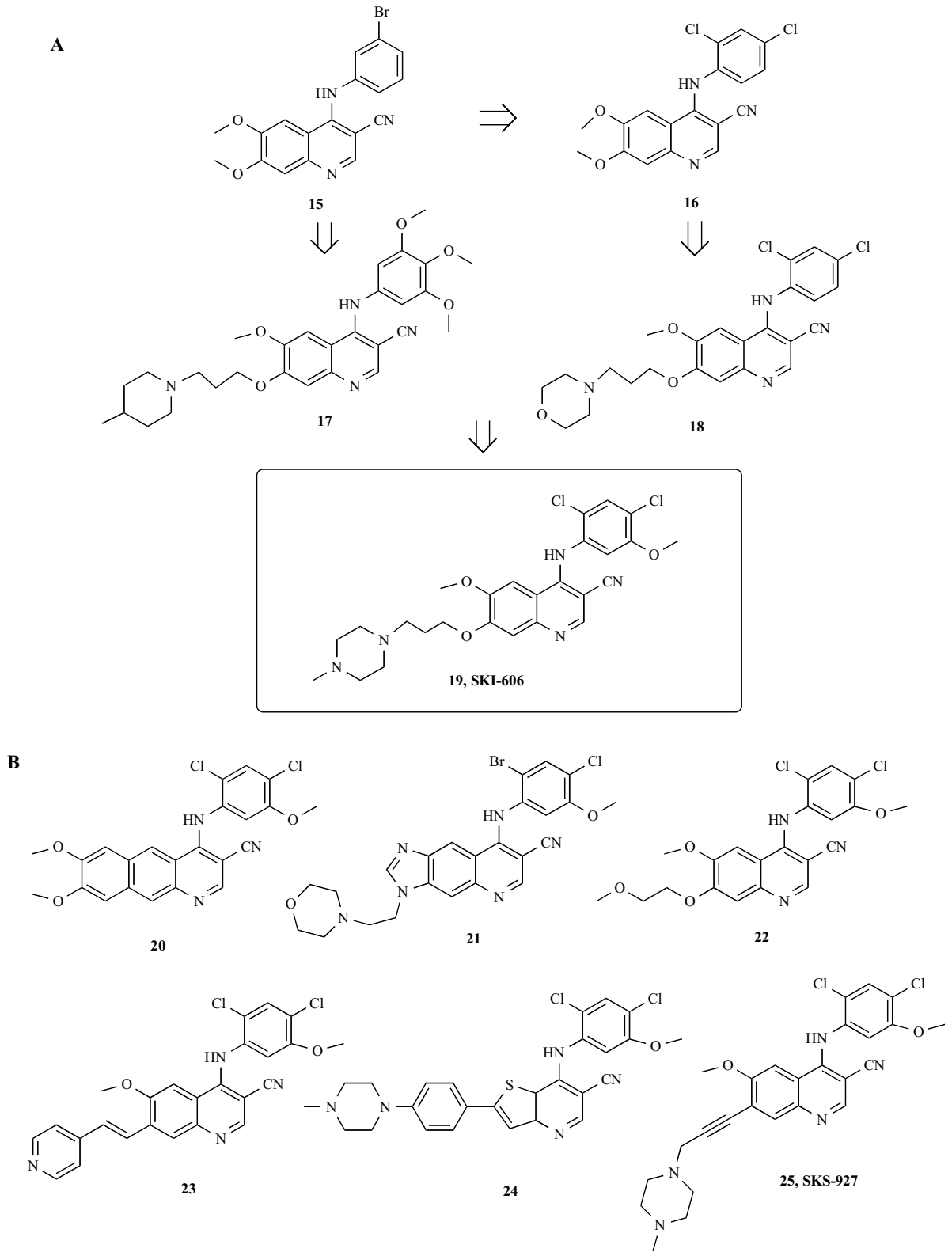


Fig. (3). 3-Quinolinecarbanitriles Src inhibitors part **A** and part **B**.

Part A. The discovery of SKI-606 (**19**).

Part B. Analogues of SKS-927 (**25**).

Table 3. The Src Kinase and Tumor Cell Inhibition Activity of Compounds 16-25

Cmpd. No.	Src, IC_{50} (nM)	Src Cell, IC_{50} (nM)	Cmpd. No.	Src, IC_{50} (nM)	Src Cell, IC_{50} (nM)
16	30	-	21	9	670
17	5.5	1300	22	1.5	370
18	3.8	940	23	4.2	80
19	1.2	100	24	13	720
20	1.4	210	25	3.9	73

tures of these compounds (**17** and **18**). When a *N*-methyl-piperazine group was brought into the skeleton, the most important compound SKI-606 (**19**) (Fig. **3**. Part A) was discovered. However, in 2003, it was found that SKI-606 (**19**) could also inhibit Abl tyrosine kinase with an IC_{50} value of 1 nM [48]. After that, a lot of analogues [49] of SKI-606 (**19**) were synthesized and evaluated, and a great deal of them showed high affinities to Src kinase and had good inhibition activities towards different cancer cell lines (Table **3**).

Some analogues of 3-quinolinecarbanitrile core structure, for example benzo[g]quinolines (**20**) [50], imidazo[4,5-g]quinoline (**21**) [51], and thieno[3, 2-*b*] pyridine (**24**) [52], also have been prepared and tested with Src kinase assays.

Recently, 7-ethynyl compounds whose side chains were substituted by ether group (**22**) [53], pyridine (**23**) [54], or phenyl piperazine (**24**) [52] that contain some water soluble groups were prepared and evaluated, and another prominent compound SKS-927 (**25**) (Fig. **3**. Part B) was discovered as an excellent Src kinase inhibitor [55].

4.5. Pyrido[2,3-*d*]pyrimidine

The scientists from Pfizer reported [56] 2-substituted aminopyrido[2, 3-*d*] pyrimidine-7-yl ureas as a novel class of soluble, potent, broadly active tyrosine kinase (TK) inhibitors. One of the thoroughly evaluated members, compound (**26**) (Fig. **4**), with IC_{50} values of 0.21 μM (PDGFR), 0.049 μM (bFGFR), and 0.018 μM (*c*-Src), was evaluated *in vivo* and produced a tumor growth delay against the Colo-205 colon xenograft model.

4.6. 2-Aminothiazole-5-carboxamides

A series of 2-heteroaromatic amino-thiazole-5-carboxamides were identified as potent Src/Abl dual kinase inhibitors by scientists from BMS through the screening of their internal compound library. The successful optimization through iterative structure-activity relationship researches identified analogs **27** (Dasatinib, BMS-354825) [57, 58] and **28** [59] with IC_{50} values of 0.4 nM and 1 nM respectively in a *pan*-Src inhibition test. They also showed excellent anti-proliferative activities against hematological and solid tumor cell lines. Now BMS-354825 (**27**) (Fig. **4**) is in the clinical trials for the treatment of chronic myelogenous leukemia.

4.7. Benzotriazines Derivatives

In the recent years, scientists from TargeGen reported [60, 61] the discovery of a new series of inhibitors towards Src kinase with novel benzotriazine structures. By designing, synthesizing and optimizing many kinds of derivatives, compounds (**29**) and (**30**) (Fig. **5**) were identified as the most potent compounds with the IC_{50} values of 6 nM and 11 nM respectively, and demonstrated good activities in different human tumor cell lines.

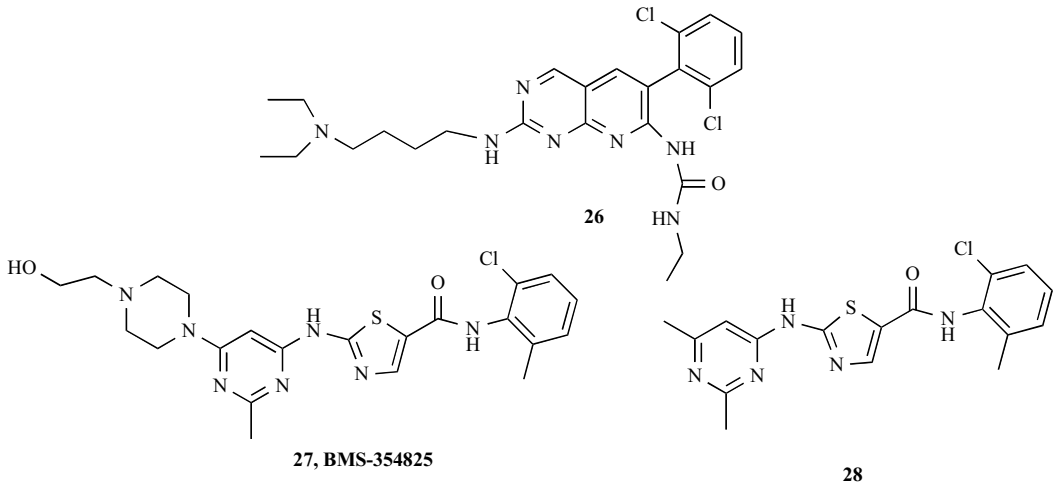


Fig. (4). BMS-354825 (**27**) and its analogues.

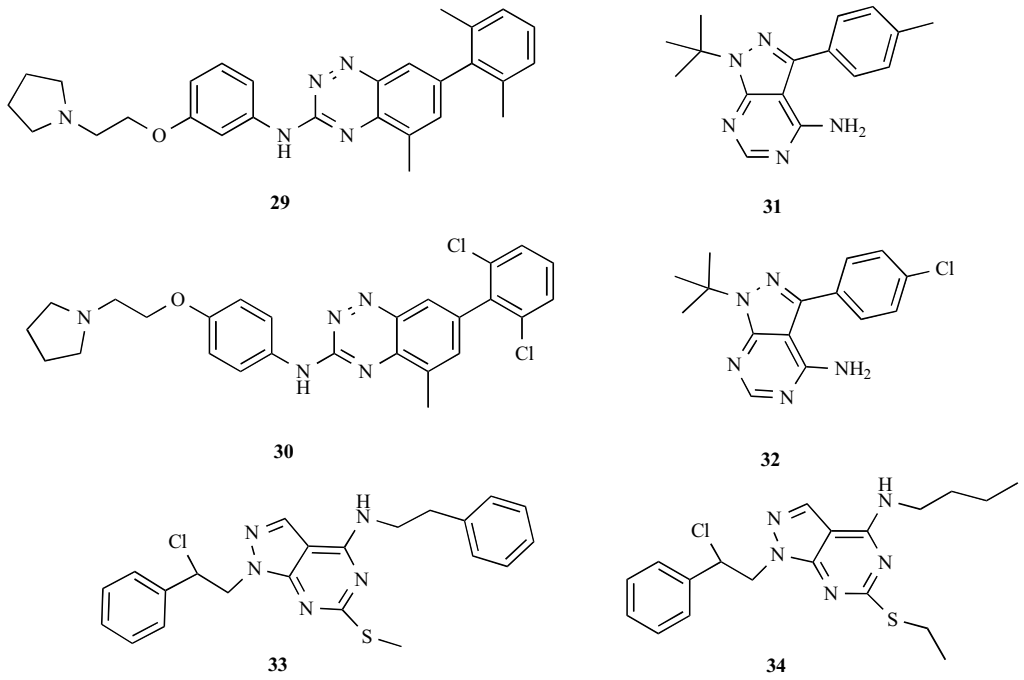


Fig. (5). The structures of benzotriazines and pyrazolo[3,4-*d*]pyrimidines derivatives Src inhibitors.

4.8. Pyrazolo[3,4-*d*]pyrimidines

PP1 (**31**) and PP2 (**32**) (Fig. **5**) discovered by Pfizer, pyrazolo[3,4-*d*]pyrimidine derivatives, belong to traditional ATP analogues which could inhibit Lck and Fyn with the IC_{50} values around 5 nM *in vitro* [62, 63]. Fabio Carraro and Silvia Schenone etc, further optimized [64, 65] this series of compounds and discovered some new inhibitors of Src kinase, such as compounds (**33**) and (**34**), which could significantly reduce 8701-breast cancer cell proliferation with the IC_{50} values of 31 μM and 38 μM, respectively.

4.9. Indolin-2-one Derivatives

SU5416 (**35**) and SU6668 (**36**) (Fig. **6**) were specified VEGFR inhibitor derived from indolin derivatives that developed by Sugen company [66, 67]. In the recent years, some novel substituted indolin-2-ones were also discovered

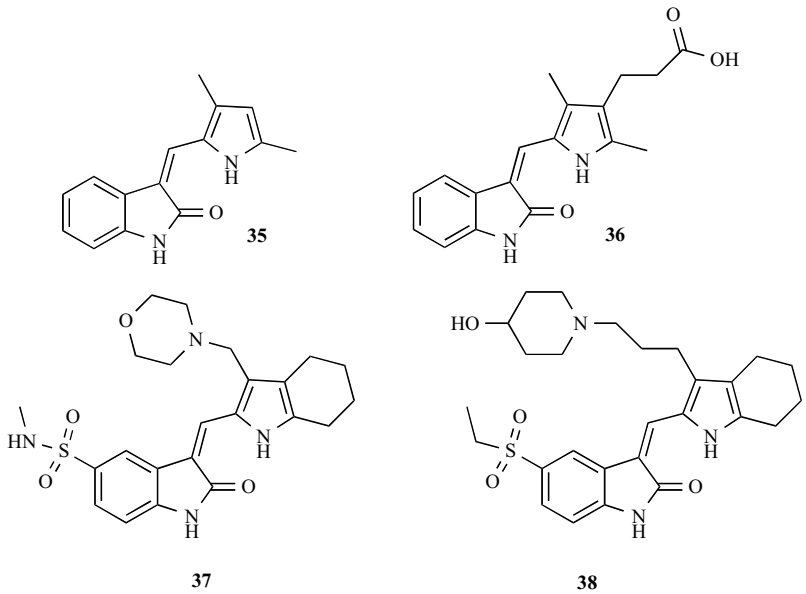


Fig. (6). The structures of indolin-2-one derivatives Src inhibitors.

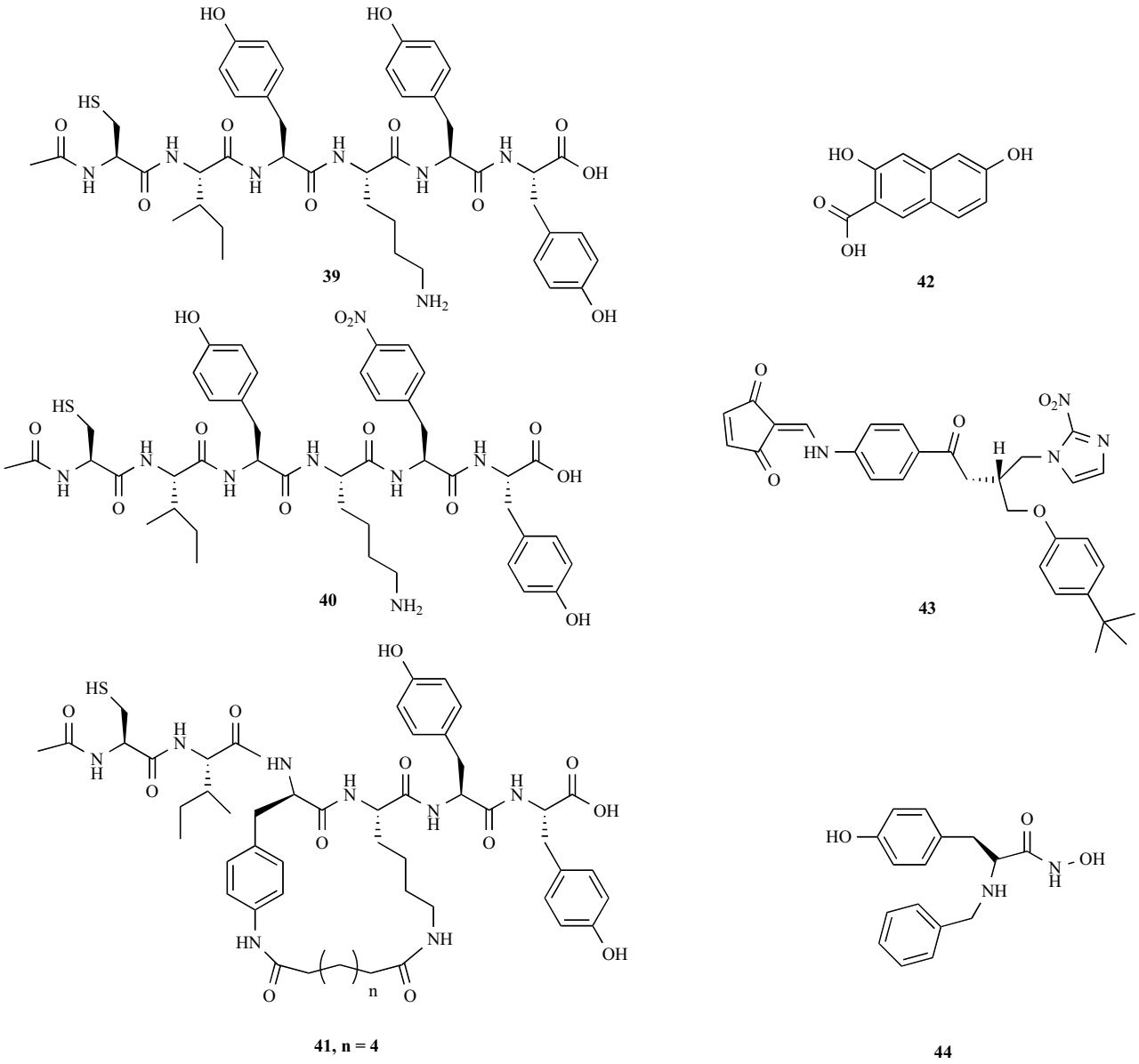


Fig. (7). The peptides and some other Src kinase inhibitors.

as potent non-receptor tyrosine Src kinase inhibitors [68, 69]. Compounds (**37**) and (**38**) inhibited Src with IC_{50} values of 10 nM and 70 nM, which were about 6-8 folds more potent than those to VEGFR.

4.10. Peptides and their Analogues

Some researches of peptide analogues have also been made as the Src kinase inhibitors. But generally speaking, peptides are poor inhibitors to Src kinase domain (SH1) for their weak affinities to the active site of SH1 [70]. The peptide CIYKYY (**39**) was reported [71] as a potent inhibitor of Src phosphorylation of YIYGSFK in a kinase assay at a relatively high concentration (IC_{50} = 400 μM), then a new series of peptide analogues of Ac-CIYKYY were synthesized by functional group modifications in the side chains or by introducing conformational constraints, to improve the inhibition potency against Src kinase [72]. Ac-CIYKF(4-NO_2)Y (**40**, IC_{50} = 0.53 μM) and conformationally constrained peptide **41** (IC_{50} = 0.28 μM) exhibited 750- and 1400-folds higher inhibition activities respectively, compared with compound (**39**) (Fig. **7**). Though the inhibition activities of the peptides were elevated, their inhibition mechanism against *c*-Src remains unknown.

4.11. Others

Besides the potent structures stated above, there have been some other novel skeletons that were used as Src inhibitor scaffolds. For example, some hydroxynaphthalene derivatives (**42**) (Fig. **7**) [73], 2-methylene-4-cyclopentene-1, 3-dione derivatives (TX-2036, **43**) [74] and even some hy-

droxamate derivatives (**44**) [75] were designed and synthesized as Src inhibitors. But these compounds were not so potent towards Src or did not have good selectivities compared with the quinoline derivatives.

5. CHALLENGES WITH Src INHIBITORS

Since the year 2000, there have been many different kinds of Src inhibitors emerged, including the kinase inhibitors, SH2 and SH3 inhibitors. In each strategy, some drug candidates were discovered. This has confirmed that the Src tyrosine kinase is an attractive target for drug design.

But there are still some problems waiting medicinal chemists to solve. The first puzzle is how to achieve the selectivity to Src kinase. The overall structural organizations as well as the SH1, SH2, and SH3 domains are very similar among all PTK members, though there are indeed some slight differences among the enzymes. For example, different 4-anilines of the quinoline and quinazoline derivatives demonstrate different enzyme affinities to the subfamilies of PTKs. However, the selectivity is not absolute to all enzymes. SKI-606 (**19**) was identified as Src inhibitor initially, but in accompaniment with the oncology clinical research, it was also found to be an Abl inhibitor. According to some recent researches, in addition to the Src kinase, dasatinib (BMS-354825, **27**) also binds to EGFR [76]. In fact, when comprehending all these inhibitors stated above, the nature of the selectivity among different kinases depends on the microcosmic structure of the kinases. So some dual inhibitors may be the right answers in tumor therapy. The genesis of tumors and leukemia are so complicated, that if a Src inhibitor is selective and potent, it is welcome; but if it inhibits some other PTKs at the same time, which are also overexpressed in tumors, the compound may own some superpower compared with the sole inhibitors.

The drug resistance is another hot topic in cancer therapy. Many patients in advanced stage cancers well responded with Glivec initially but then relapsed [77]. Various mechanisms have been proposed for this resistance, including mutations in the Src kinase domain. Some recent research results reveal a complex effect of Src on cellular drug responses and provide an explanation for the effect of this oncogene on cellular drug resistance [78]. The specific mechanism of the drug resistance is out of the scope of this paper, but this may also support that multiple target inhibitors may be necessary to overcome the acquired resistance in Src dependent leukemia. Some similar paradigms may also exist in the treatment of Src dependent colon cancer and EGFR-dependent lung cancer.

CONCLUSIONS

It has been a long period since the discovery of the *src* gene. Studies on SFKs have led to new insights into the role of tyrosine phosphorylation in cell physiology, the signalling pathways by which cell surface receptors regulate cell growth and proliferation, and the function of modular domains that mediate protein–protein interactions. Great efforts to design and synthesize small molecular Src inhibitor drugs for cancer research have been made in recent years. Although there are no Src inhibitors entering the market till now, several promising compounds have been under strict clinical therapeutic trials for anticancer treatment.

In the near future, research of Src kinase inhibitors will still be an attractive area because their inhibition might influence several important characteristics of tumorigenesis. With the advantage of high technology utilized in drug research, including crystallization of protein kinases, models for the binding of lead structures at the ATP-binding site, computer aided *de novo* drug design, combinatorial chemistry, parallel synthesis, high-throughput screening to optimize the promising lead compounds, rapid 3D screening of large compound libraries and fast evaluation of compound properties by new ADME models, the hitting ratio of new Src inhibitors will be elevated in various stages of drug R&D, and the promising results of their clinical trials will also appear in the near future.

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ABBREVIATIONS

EGF	=	Endothelial growth factor
EGFR	=	EGF receptor
FGFR	=	Fibroblast growth factor receptor
MAPKK	=	Mitogen-activated protein kinase kinase
PDGFR	=	Platelet-derived growth factor receptor
PTK	=	Protein tyrosine kinases
RPTKs	=	Receptor protein tyrosine kinases
RSV	=	Rous sarcoma virus
SFKs	=	Src family of protein tyrosine kinases
SH2	=	Src homology 2
SH3	=	Src homology 3
VEGF	=	Vascular endothelial growth factor
VEGFR	=	VEGF receptor

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