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FAK inhibitors as promising anticancer targets: present and future directions

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FAK, a nonreceptor tyrosine kinase, has been recognized as a novel target class for the development of targeted anticancer agents. Overexpression of FAK is a common occurrence in several solid tumors, in which the kinase has been implicated in promoting metastases. Consequently, designing and developing potent FAK inhibitors is becoming an attractive goal, and FAK inhibitors are being recognized as a promising tool in our armamentarium for treating diverse cancers. This review comprehensively summarizes the different classes of synthetically derived compounds that have been reported as potent FAK inhibitors in the last three decades. Finally, the future of FAK-targeting smart drugs that are designed to slow down the emergence of drug resistance is discussed.

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Keywords: cancer • FAK inhibitors • focal adhesion kinase • PROTACs

Cell migration performs significant roles not only in diverse biological processes, such as embryonic development, but also in various diseases, including fibrogenic disorders and cancer [1–3]. Cell migration involves several steps, including leading-edge protrusion, focal adhesions turnover, tractional forces generation, and tail retraction and detachment [4,5]. At each cell migration step, the integrin family, as cellular receptors for the extracellular matrix (ECM), is fundamental for cell adhesion [6]. Integrins can regulate cell migration and functions, since they can cocluster with several signaling molecules in focal adhesions. Several proteins are located in the focal adhesions, such as filamin, fibronectin, integrin, talin and FAK. FAK, also known as protein tyrosine kinase 2 (PTK2), is a nonreceptor tyrosine kinase with a weight of approximately 125 kDa. FAK is one of the most essential and the earliest identified signaling molecules. FAK was given its name in 1992 when discovered in the cell adhesion area near v-Src transformed chick embryo [3,7–10]. The interactions of FAK with growth factor receptors and integrins contribute to cell anchorage dependency, invasive growth, and motility, which are correlated with malignant overgrowth. The human FAK protein is comprised of central kinase domain, which contains the activation loop (2 tyrosine residues: Tyr576 and Tyr577) that can be phosphorylated by Src and is required for FAK kinase activity. This middle domain is flanked by an amino-terminal FERM (band 4.1-ezrin-radixin-moesin) domain that interacts with growth factor receptors and integrin (Figure 1). The third domain is a carboxy-terminal focal adhesion targeting (FAT) sequence, which obliquely connects FAK to integrins and contains Tyr925, a key phosphorylation site [11,12]. The FAT region as a tetra-helical structure provides binding of FAK to the focal adhesion proteins paxilin and talin, a

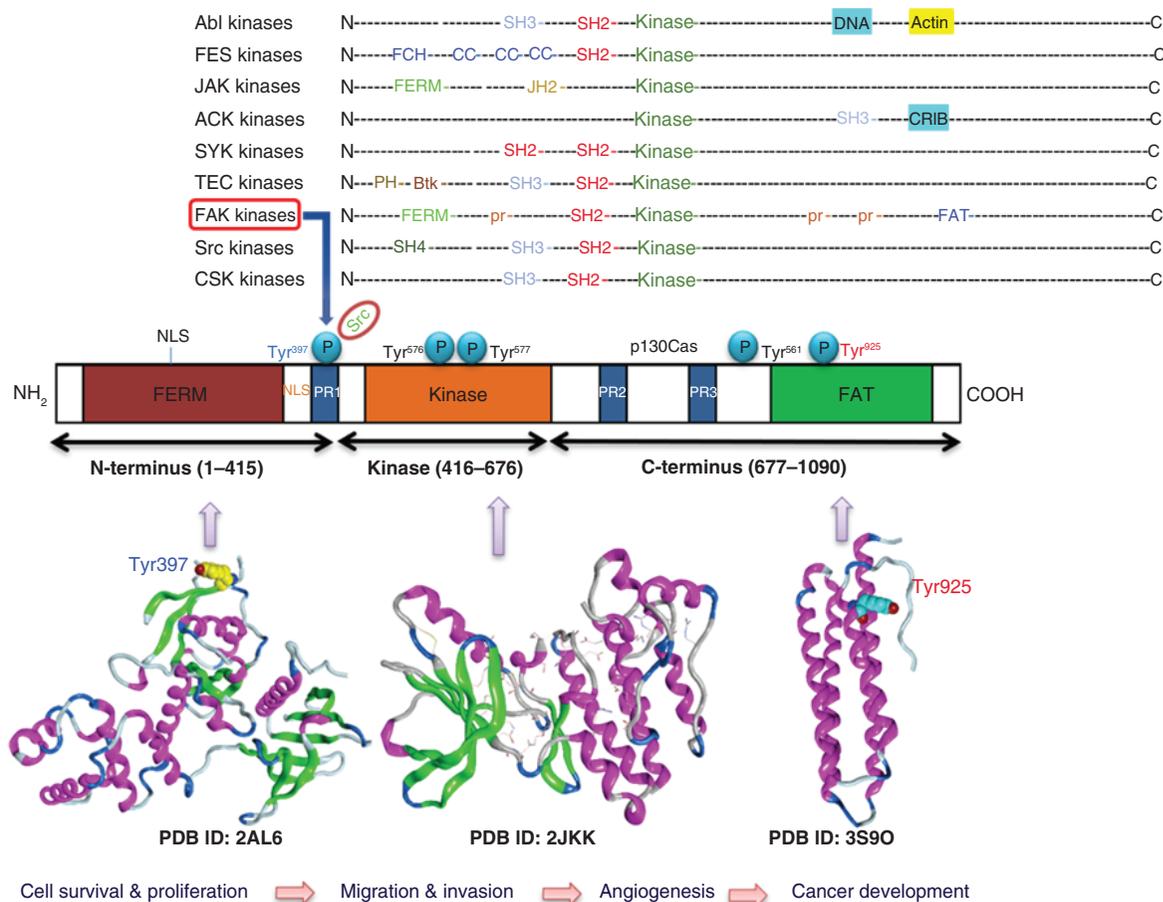


Figure 1. The FAK structure.
PDB: Protein Data Bank.

necessary process for cell adhesion. FAK includes three regions rich in proline (PR1-3). Their role is to mediate binding with various molecular effectors and regulators, such as Src homology 3 (SH3)-domain-containing proteins (Figure 1) [13]. FAK is capable of enhancing cell survival in a kinase-independent manner, since FAK FERM domain forms phosphorylation-independent complexes with different proteins [14,15].

FAK activity is described as the ability of this tyrosine kinase to transfer a phosphate group from ATP onto site-specific tyrosine residues in substrate proteins such as p130Crk-associated substrate (CAS), paxillin, cortactin, Src, VE-cadherin, α -actinin and β -catenin as a means of protein function alteration [16]. But, like other kinases, FAK's activity is spatiotemporally regulated by other cellular proteins and events. For example, the interactions of FAK with membrane receptors such as integrins are assumed to induce FAK conformational switch to expose Tyr397 phosphorylation sites [17]. This is accomplished through the binding of FERM to phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂], leading to transient FERM: FERM and FERM: FAT dimerization, allowing FAK autophosphorylation at Tyr397. FAK Tyr397 phosphorylation generates a binding site for the SH2 domain of Src [11,18]. FAK phosphorylation at Tyr397 can elevate the binding of FAK and the phosphorylation of acceptor proteins (Src), which play a fundamental role in cell migration. This pTyr397 affords a docking site for the acceptor protein Src, causing phosphorylation of additional sites on FAK enzyme and creating a high-affinity active site for SH2 domain of Src [19,20]. This FAK-Src complex was found to be crucial in the FAK-associated signaling process. Moreover, the binding of Src with FAK-Tyr397 residue leads to phosphorylation of two residues, Tyr576 and Tyr577, placed on FAK catalytic site and Tyr861 residue required for total catalytic activity. When FAK activates Src, various signal transduction pathways are stimulated, including PI3K-Akt, Rho/Rac/PAK, and RAF/JNK, leading to cell mobility and cell survival modulation [21,22]. FAK hyperphosphorylation and overexpression are directly associated with several kinds of solid tumors [23-25]. FAK overexpression can boost cell motility and

survival as it contributes to tumorigenicity and metastasis. FAK hyperactivity improves the resistance of tumor cells to traditional anticancer therapy [26]. The N- and C-terminal sequences of FAK produce an autoinhibitory structure, and caspase cleavage of FAK results in the removal of this autoinhibition. Subsequently, FAT domain is released, leading to the dephosphorylation of FAK and induction of cell death [11,27]. Epithelial-to-mesenchymal transition (EMT) is a functional transition accompanied by multiple biochemical changes of epithelial cells during embryogenesis, organ development and repair, and various pathological processes (such as tumor invasiveness and metastasis). Overexpression of FAK was also found to be associated with tumor invasiveness and metastasis characteristics of the EMT process.

At the molecular level, the association of VE-cadherin and FAK is mediated by FERM domain binding to the VE-cadherin cytoplasmic domain. Activation of FAK at both adherens junctions (protein complexes that form epithelial and endothelial cell–cell interactions) and adhesions likely promotes crosstalk between cadherin receptors and integrin [28]. Previous studies revealed that genetic inhibition of FAK in endothelial cells lessened β -catenin and VE-cadherin phosphorylation and prevented metastasis and extravasation of tumor cells [29,30]. Additionally, many studies have shown the crucial role played by FAK enzyme in regulating adhesion dynamics. Intracellular Ca-dependent proteolysis mediated by calpain family members was proposed to regulate cell migration by modulating adhesion dynamics. Focal adhesion proteins such as FAK, talin and paxillin are targets for calpain family, and these targets' proteolysis was found to contribute to regulating both adhesion dynamics and cell migration [31]. FAK also augments the rate of proteosomal degradation of the tumor suppressor p53; this has been proposed as a key mechanism by which FAK promotes cell survival under stressful growth conditions [32–34]. FAK binds to and defends nucleostemin, a protein that binds to p53, regulates cell cycle and cell differentiation, and is a marker for cancer stem cells from stress-induced degradation [15,35]. In addition to promoting survival, FAK also suppresses apoptosis by binding to the death domain of receptor-interacting protein (RIP). Such binding leads to cell survival, enhanced cell adhesion, migration and promotion of cell cycle [36]. Tumor cells subjected to FAK attenuation experience apoptosis mediated by Fas-associated death domain protein (FADD) and caspase-8 [37,38].

Over the past three decades, evidence has solidified the role of FAK in anchorage-independent growth of tumor cells; FAK emerged as a central mediator of not just integrin signaling but also for signaling downstream for other classes of cell surface receptors [3,39]. FAK-dependent regulation of cell adhesion and migration has been established in several cell types and is widely accepted as a pathway that contributes to cancer pathogenesis and other diseases [40,41]. Consequently, FAK is regarded as a promising target for cancer treatment. Indeed, various small anti-FAK molecules have already been developed by several pharmaceutical companies for clinical trials [42–47]. Favorably, FAK inhibition affects other vital proteins, such as signal transducer and activator of transcription 3 (STAT3) and Akt. STAT3 plays a fundamental role in cell growth, and is found to be activated mostly in solid tumors (as with FAK) [48–51]. Fortunately, inhibition of FAK leads to STAT3 reduction, resulting in apoptosis induction and potent antitumor activity [52–54]. Moreover, Akt, a protein kinase, plays an important role in regulating various cellular functions such as metabolism, proliferation, survival, growth and protein synthesis [55,56]. In this respect, inhibition of Akt phosphorylation leads to significant anticancer activity. Interestingly, FAK inhibition results in Akt phosphorylation suppression [57–61]. Also, inhibition of FAK induces cell cycle arrest at G2/M phase and considerably reduces S phase. This effect is accompanied by increasing early apoptotic cells [62,63]. FAK inhibition may lead to P53 activation that subsequently leads to cell cycle arrest and apoptosis induction [64,65].

Several cancer cells express FAK protein to promote cell survival, proliferation and metastasis, which makes FAK enzyme a promising target for cancer management. These cells comprise several types of cancers such as pancreatic cancer cells [66,67], colon cells [68–70], hepatocellular carcinoma cells [62,71], lung cancer cells [72–74], ovarian cancer cells [61,75], renal cancer cells [76–78], breast cancer cells [79–82], cervical cancer [83] and prostate cancer cell lines [84]. This wide variety of cancer cells that express FAK protein shows that targeting this kinase is inevitably promising in the fight against cancer.

Binding site of FAK

The most studied and targeted site in FAK protein is the ATP binding site; this site was taken up by several research groups and was targeted using different scaffolds of FAK inhibitors. The ATP binding site of the FAK protein contains specific characteristics that allow various FAK inhibitors to bind identically. FAK inhibitory activity inside the ATP binding site depends mainly on two pivotal sites: the kinase hinge region that comprises mainly Cys502 amino acid and the helical DFG motif of the activation loop (Asp564, Phe565 and Gly566 amino

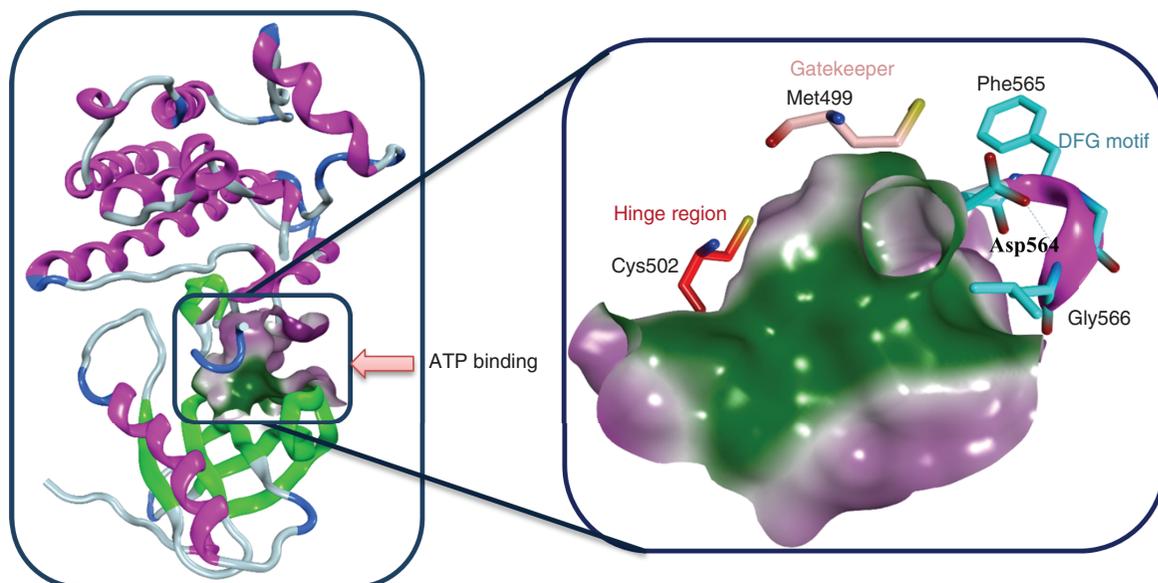


Figure 2. The ATP binding site of FAK including the key interaction sites: hinge region and DFG motif.

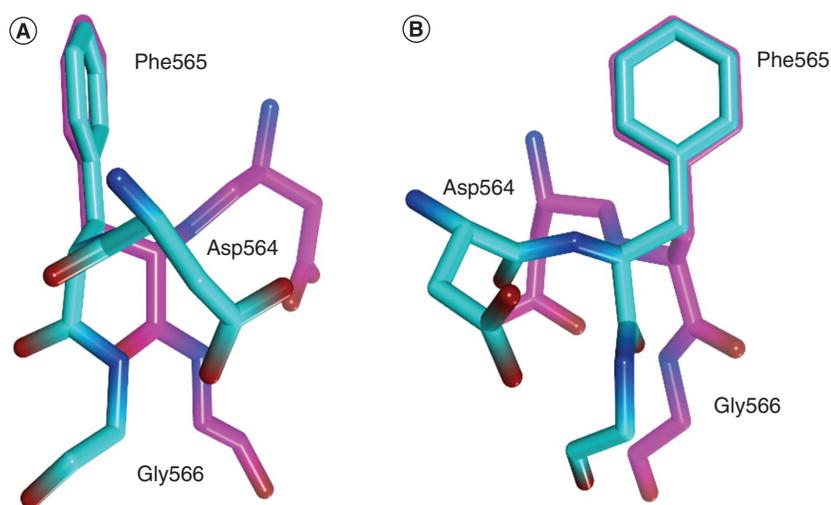


Figure 3. Superimposition of the DFG motif illustrates the conformational change of Asp564 in FAK bound to TAE226 (cyan) compared with Asp564 in the natural FAK domain kinase (magenta). (A) Side view and (B) front view.

acids; Figure 2). Compared with the FAK domain, the phi torsion angle rotates Asp564 of the DFG motif by 113° upon administration of different FAK inhibitors, such as TAE226 and PF562271 (Figure 3) [85,86]. Interestingly, this unusual helical DFG conformation is essential to obtain optimum anti-FAK activity. The upper part of the active site includes the gatekeeper residue Met499, while the lower part contains a solvent region. The active site contains some important amino acids that are usually involved in electrostatic interactions with different FAK inhibitors such as Arg426, Ile428, Ala452, Glu500, Leu501, Gly505, Leu553 and Leu567 (Figure 4). Among these various amino acids, Cys502 of the kinase hinge and Asp564 of the DFG motif are considered the key residues within the ATP binding site of FAK that should be targeted to obtain potent FAK inhibitory activity.

FAK inhibitors

The past three decades saw a significant increase in the development of new FAK inhibitors. Several research groups have worked to design appropriate scaffolds to provide the best anti-FAK activity. In the next paragraphs, the authors review the most promising findings and the status of several drugs in their clinical trial development.

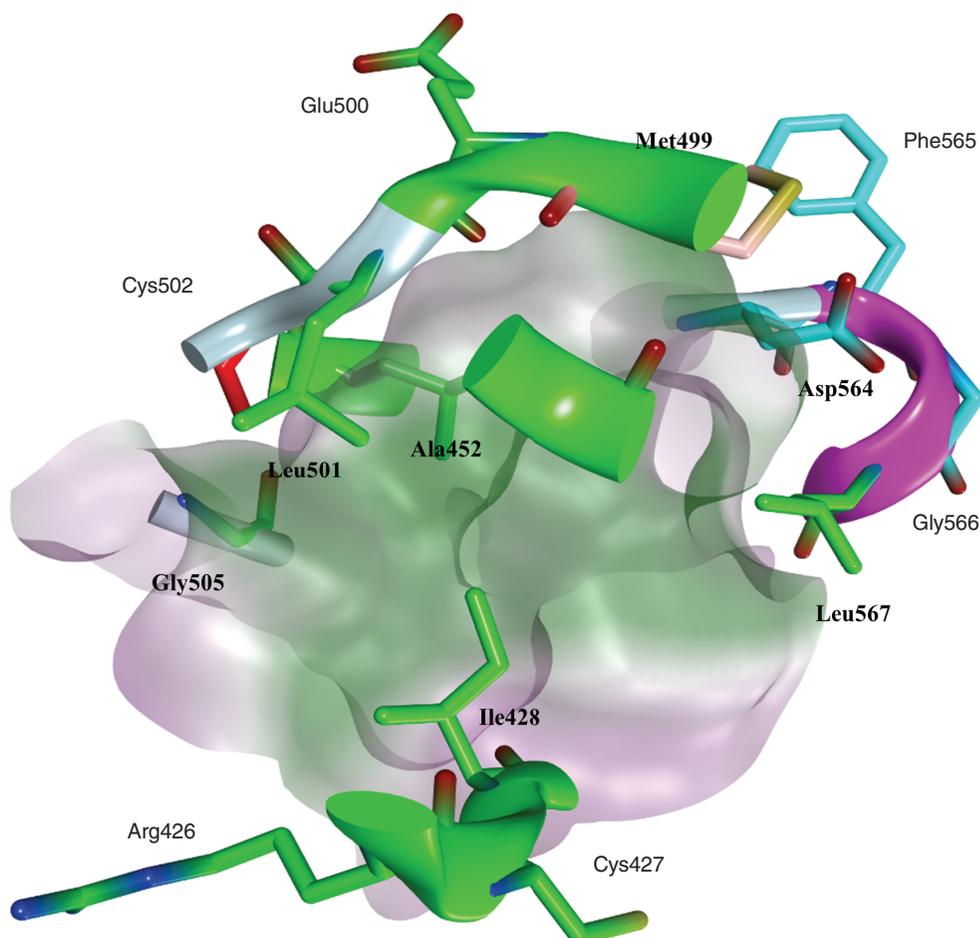


Figure 4. The ATP binding site of FAK with its key amino acids.

Various active sites in the FAK protein have been investigated in the development of anticancer agents. The majority of attempts have been directed toward targeting the ATP binding site of the kinase domain, whereas some attempts have targeted the allosteric binding site (Figure 5). Numerous computational approaches have targeted FAK-FERM and FAK-FAT domains to prevent phosphorylation at Tyr397 and Tyr925 amino acids, respectively (Figure 5). FAK inhibitors are classified as follows: six-membered heterocyclic FAK inhibitors, five-membered heterocyclic FAK inhibitors, allosteric FAK inhibitors, FAK-FAT domain inhibitors, FAK-FERM domain inhibitors, FAK degradation through proteolysis targeting chimeras (PROTACs) and miscellaneous FAK inhibitors.

FAK inhibitors

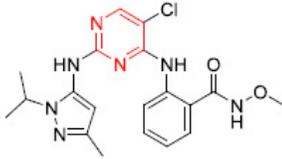
Six-membered heterocyclic FAK inhibitors

One of the most well known FAK inhibitors is TAE226 (**1**, Table 1), which has shown significant FAK inhibitory activity with an IC_{50} value of 5.5 nM [86]. This inhibitory effect resulted in superior antiproliferative activity in different cancer cell lines. Compound **1** thoroughly inhibited invasion and proliferation in glioma cells in both *in vitro* and *in vivo* studies [87]. It induced apoptosis in BT474 and MCF-7 breast cancer cells, inhibited tumor growth, and reduced its weight profoundly in HeyA8 ovarian cancer cells [88,89]. Consequently, **1** decreased cell viability, induced apoptosis and arrested cell cycle at G2 phase in human neuroblastoma cells [90]. Compound **1** also had a remarkable anticancer effect on pancreatic carcinoma, squamous cell carcinoma and colorectal cancer, but it did not enter clinical trials due to severe side effects, since it affects glucose blood levels (inhibits insulin receptor) and metabolism [91–94]. Compound **1** was cocrystallized in the ATP binding pocket of the FAK enzyme (PDB codes: 4D58, 4D4S, and 2JJK) [86,95]. The cocrystallized orientation of **1** revealed the formation of two hydrogen bonds between the nitrogen of pyrimidine and *o*-methoxy aniline moieties and the carbonyl oxygen of Cys502 of

Table 1. Preclinical/clinical trial FAK inhibitors.

No.	Compound name	Structure	FAK (IC ₅₀ , nM)	Preclinical/clinical phase	Target cancer cell
1	TAE226		5.5	Preclinical	GIST
2	PND-1186 (VS-4718)		1.5	Phase I	Pancreas
3	PF-573228		4	Preclinical	Breast and lung
4	PF-562271		1.5	Phase I	Pancreas, head, neck and prostate neoplasms
5	CEP-37440		2	Phase I	Solid tumors
6	Defactinib (VS-6063)		0.6	Phase II	Non-small cell lung cancer

Table 1. Preclinical/clinical trial FAK inhibitors (cont.).

No.	Compound name	Structure	FAK (IC ₅₀ , nM)	Preclinical/clinical phase	Target cancer cell
7	GSK-2256098		Ki (0.4 nM)	Phase II	All cancers, neoplasms
–	BI-853520 (IN-10018)	Not disclosed structure	1	Phase I	Neoplasm

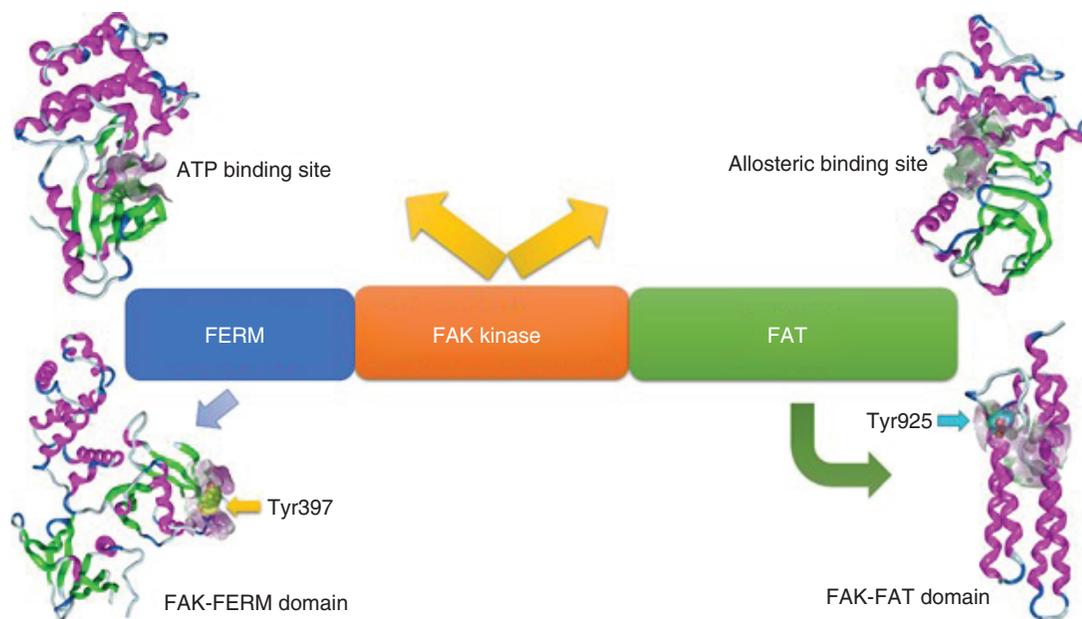


Figure 5. Methods for targeting FAK protein in the development of anticancer agents.

the kinase hinge (Figure 6). Two hydrophobic contacts were observed between the carbons of the pyrimidine ring and Leu553 and Ala452 amino acids. The chloro group on C5 of the pyrimidine ring penetrated deep into the kinase binding pocket to be positioned near the gatekeeper amino acid Met499. The carbonyl group of the methyl carbamoyl moiety formed a hydrogen bond with the nitrogen of Asp564 of the DFG motif of the activation loop. The hydrophobic amino acid Leu567 that immediately follows the DFG sequence made a hydrophobic interaction with the 4-aniline ring.

PND-1186 (VS-4718, **2**) was designed as a selective FAK inhibitor (IC₅₀ = 1.5 nM) with superior phosphorylation inhibition at Tyr397 of FAK. It has the same structural features as **1**; however, the pyridine moiety was introduced instead of pyrimidine, and the chlorine was replaced by trifluoromethyl as a strong electron withdrawing group. These structural modifications resulted in 3.5 times more anti-FAK activity than **1** [96]. Compound **2** revealed a strong ability to inhibit tumor growth and metastasis in 4T1 and MDA-MB-231 breast cancer cells and inhibited orthotopic ID8-IP ovarian cancer growth [75,97]. *In vitro*, **2** decreased the expression IL-6, which is induced by tumor necrosis factor- α , suggesting that compound **2** inhibited the progression of tumors by connecting stromal cells and tumor cells. Compared with gemcitabine, **2** profoundly extended the survival of early and late KPC mice with pancreatic ductal adenocarcinoma (PDAC) progression [98]. Compound **2** overcame PDAC fibrosis and immunosuppressive cell population; thus, immune surveillance increased significantly. Compound **2** reversed the multidrug resistance mediated by the ATP-binding cassette (ABC) transporters including ABCB1 and ABCG2 (ABC transporters decrease the intracellular concentration of different anticancer agents by pumping the anticancer compound out of the tumor cell, which is considered a drastic mechanisms in multidrug resistance) [99–103]. The combination of **2** with doxorubicin or paclitaxel considerably enhanced the IC₅₀ values in

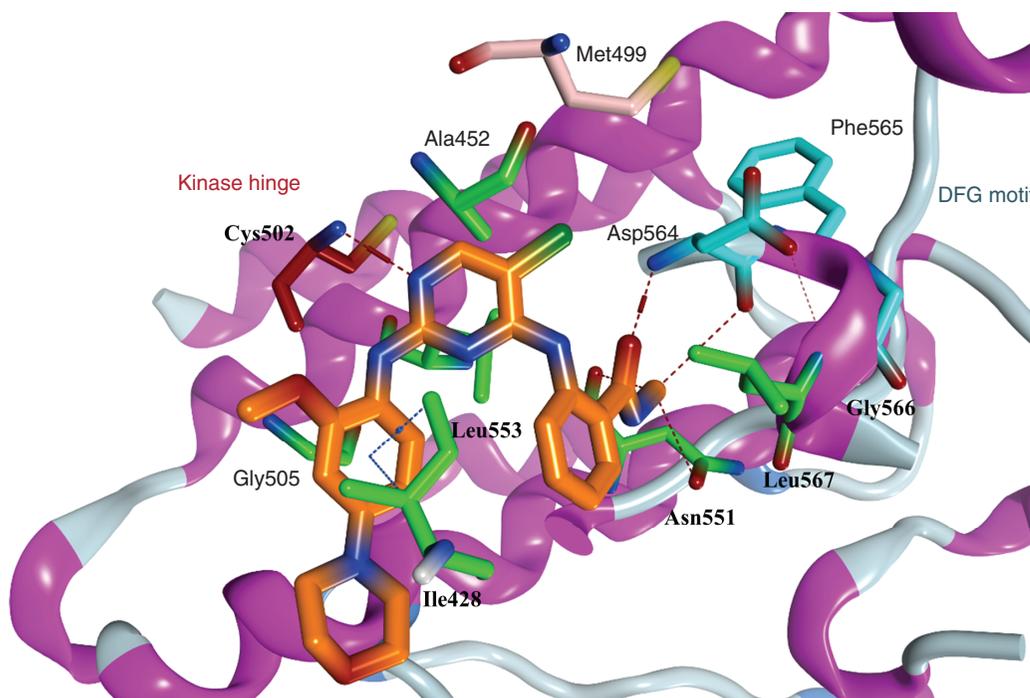


Figure 6. Cocrystallized structure of TAE226 (orange) inside the ATP binding site of FAK, Protein Data Bank code: 2JKK.

KB-C2 and SW620/Ad300 (ABCB1-overexpressing resistant cells) in a dose-dependent manner. Despite these significant results, clinical trials of **2** were terminated and the compound was withdrawn (ClinicalTrials.gov Identifier: NCT01849744 and NCT02215629).

Another inhibitor bearing trifluoromethyl moiety (PF-373228, **3**) was investigated due to its high specificity toward FAK ($IC_{50} = 4$ nM). Notably, the 4-morpholinophenyl group, found in **1** and **2**, was replaced by a dihydroquinolinone moiety, and methylsulfonyl amine was introduced into the benzene ring. Despite possessing strong FAK inhibitory activity, compound **3** did not display promising apoptosis induction or proliferation inhibition [104]. PF-562271 (compound **4**), the same scaffold as PF-373228 (**3**, Table 1), is a reversible chimeric inhibitor of proline-rich tyrosine kinase 2 (Pyk2: A cytoplasmic tyrosine kinase within the FAK subfamily contributes in multiple signaling pathways which regulate proliferation, migration and survival of cells) and FAK ($IC_{50} = 13$ and 1.5 nM, respectively) [85]. *In vivo*, compound **4** was able to inhibit FAK phosphorylation in a nanomolar dose-dependent manner. Moreover, the *in vivo* experiments revealed no morbidity, mortality or weight loss. Treating prostate cancer cells PC3M-luc-C6 in a mouse xenograft model with 25 mg/Kg of compound **4** resulted in 62% tumor growth inhibition in two weeks and showed a substantial ability to decrease metastasis [105]. Phase I clinical trials revealed that compound **4** is highly tolerable at 125 mg dose b.i.d. with food and is cleared via CYP 3A-mediated hepatic metabolism (positive metabolic response) [106]. Compound **4** was cocrystallized in the ATP binding site of FAK (PDB code: 3BZ3) and was oriented approximately the same as **1**; thus, the same interactions were observed [85]. The only difference was that the oxygen atom of the amino-oxindole moiety extended out of the active site cleft to form a hydrogen bond with Arg426 amino acid.

Another highly potent derivative, CEP-37440 (**5**), completed phase I clinical trials with considerable anti-FAK activity ($IC_{50} = 2$ nM) [107,108]. Compound **5** showed a high selectivity on anaplastic lymphoma kinase (ALK) with an IC_{50} value of 3.1 nM. (ALK is an orphan receptor tyrosine kinase constitutively activated in a number of cancer types.) [109] The benzocycloheptyl ring helped to improve anti-FAK activity and maintain ALK selectivity. Moreover, **5** exhibited significant dose-dependent antitumor activity in the oral dose xenograft model. Compound **5** showed good oral bioavailability and favorable *in vitro* absorption, distribution, metabolism, and excretion (ADME) properties in three different species (CD-1 mouse, cynomolgus monkey and Sprague-Dawley rat). Defactinib (VS-6063, **6**) is a strong FAK inhibitor, and its structural features showed a successful replacement of the phenyl ring (compounds **1**, **2**, **3** and **5**) or the pyridine (compound **4**) with a substituted pyrazine moiety. Compound **6**

showed the addition of benzamide moiety instead of the morpholine (compounds **1** and **2**) and δ and β lactam rings (compounds **3** and **4**, respectively). Phase I clinical trials revealed that compound **6** was well tolerated when administered in combination with paclitaxel, with no dangerous side effects [110]. Combining defactinib with gemcitabine and pembrolizumab in patients with PDAC also revealed a substantial tumor regression. This combination was estimated to be well tolerated orally with few side effects [111]. Unfortunately, Phase II clinical trials showed that compound **6** alone possessed a modest activity on *KRAS* mutant non-small cell lung cancer [112]. These results suggest that future defactinib studies focusing on multidrug regimens are warranted.

GSK-2256098 (**7**), a highly selective FAK inhibitor bearing 1-isopropyl-3-methyl-1*H*-pyrazole, showed significant Tyr397 autophosphorylation inhibition and decreased pancreatic cancer, breast cancer, neuroblastoma, and glioblastoma tumor growth significantly [113,114]. Among 261 tested kinases, **7** showed a profound selectivity on FAK inhibition. Interestingly, **7** showed 1000-times more FAK inhibitory activity than Pyk2, the nearest FAK kinase family member [113,115]. Compound **7** showed potent FAK inhibitory activity in OVCAR8 ovarian cancer, A549 lung cancer and U87MG brain cancer cell lines at IC₅₀ values of 8.5, 15, and 12 nM, respectively. Moreover, **7** exhibited variable FAK inhibitory activity in different (PDAC) cell lines; for example, it showed its best anti-FAK activity in L3.6P1, whereas no notable activity was observed in PANC-1 cells. Unlike PANC-1 cells, **7** induced apoptosis through caspase-9 PARP-related pathways and significantly decreased colony formation in L3.6P1 cell line. MTT assay revealed that **7** had a remarkable antiproliferative activity in L3.6P1 cell line (IC₅₀ = 25 μ M). It also inhibited ovarian cancer in 3D cultures and *in vivo* studies [116]. The combination of **7** with docetaxel and pazopanib resulted in a remarkable decrease in overall tumor weight [117]. Phase I clinical trial revealed that oral administration of **7** b.i.d. in a dose ranging 0.8–1.5 g resulted in 80% reduction of pFAK in patients with advanced solid tumors (melanoma and mesothelioma). Compound **7** presented an acceptable safety profile with minimal side effects, such as nausea (76%), vomiting (58%), diarrhea (65%) and decreased appetite (47%). Despite having a good safety profile, the **7**/trametinib combination showed limited support for efficacy when administering **7** b.i.d. at a dose of 500 mg and trametinib at a dose of 0.375 mg q.d. [118].

BI-853520 (IN-10018, nondisclosed structure) is a highly potent FAK inhibitor (IC₅₀ = 1 nM) that represses Tyr397 FAK phosphorylation in highly metastatic breast cancer [119]. MTS assay revealed that BI-853520 considerably reduced cell viability of PY2T, PY2T-LT and 4T1 cells grown in 2D cultures. BI-853520 inhibited growth and migration in SPC111 and SPC212 malignant pleural mesothelioma cells [120]. Western blot assay revealed a substantial ability to inhibit the FAK phosphorylation in the malignant pleural mesothelioma cells (SPC111, SPC212, M38K and P31). BI-853520 also prevented spheroid formation in P31 cells. Phase I clinical trials were performed on 21 Japanese and Taiwanese patients with different types of cancers. Unfortunately, most patients underwent disease progression (57%), while 24% showed stable disease control in various types of cancer (urachus, esophageal, meningioma, gastric cardia and intrahepatic bile duct cancer) [121]. Another Phase I clinical trial was performed on 63 patients with different types of metastatic cancers (pancreatic adenocarcinoma, ovarian cancer, esophageal cancer and soft tissue sarcoma) [122]. BI-853520 resulted in a substantial decrease in pFAK/total FAK ratio, while anticancer activity was moderate. BI-853520 showed an acceptable and manageable safety profile with favorable pharmacokinetics in both the abovementioned studies.

Recently, Ma and colleagues [44] disclosed carbonyl-substituted DPPY derivatives with potent FAK inhibitory activity. A series of nucleophilic substitution and coupling reactions were performed to obtain the target compounds. Among them, derivative **8** exhibited the highest anti-FAK activity (Figure 7). Compound **8** showed strong activity in AsPC-1, BxPC-3, PANC-1 pancreatic cancer, H1975 lung cancer and MCF-7/ADR breast cancer cell lines. The *in vivo* antiproliferative activity on AsPC-1 cells showed that **8** was able to inhibit tumor growth by 87% at a dose of 30 mg/kg. Furthermore, western blot analysis demonstrated that treating AsPC cells with **8** considerably repressed FAK phosphorylation at less than 50 nM concentration.

The same research group prepared phosphamide-containing diphenylpyrimidine derivatives (DPPY) as potent FAK inhibitors [123]. The ADP-Glo™ kinase assay revealed that FAK activity was substantially hindered when treated with the synthesized derivatives in nanomolar concentrations. Compound **9** exhibited better anti-FAK activity (IC₅₀ = 4.25 nM) than the reference TAE226 (IC₅₀ = 6.79 nM) and showed a substantial ability to inhibit the pancreatic cancer cells AsPC-1, BxPC-3 and PANC-1 (IC₅₀ = 3.31, 0.02 and 14.76 μ M, respectively) in low micromolar concentrations (Figure 7). Qu and colleagues [124] developed sulfonamide-substituted DPPY derivatives with moderate FAK inhibitory activity. Compound **10** possessed the best antiproliferative activity in cancer cells that harbor the overexpressed FAK enzyme, such as pancreatic carcinoma (AsPC-1, Panc-1, BxPC-3), leukemia (Ramos) and gefitinib-resistant lung cancer (H1975; Figure 7). The sulfonamide derivative **10** was also found

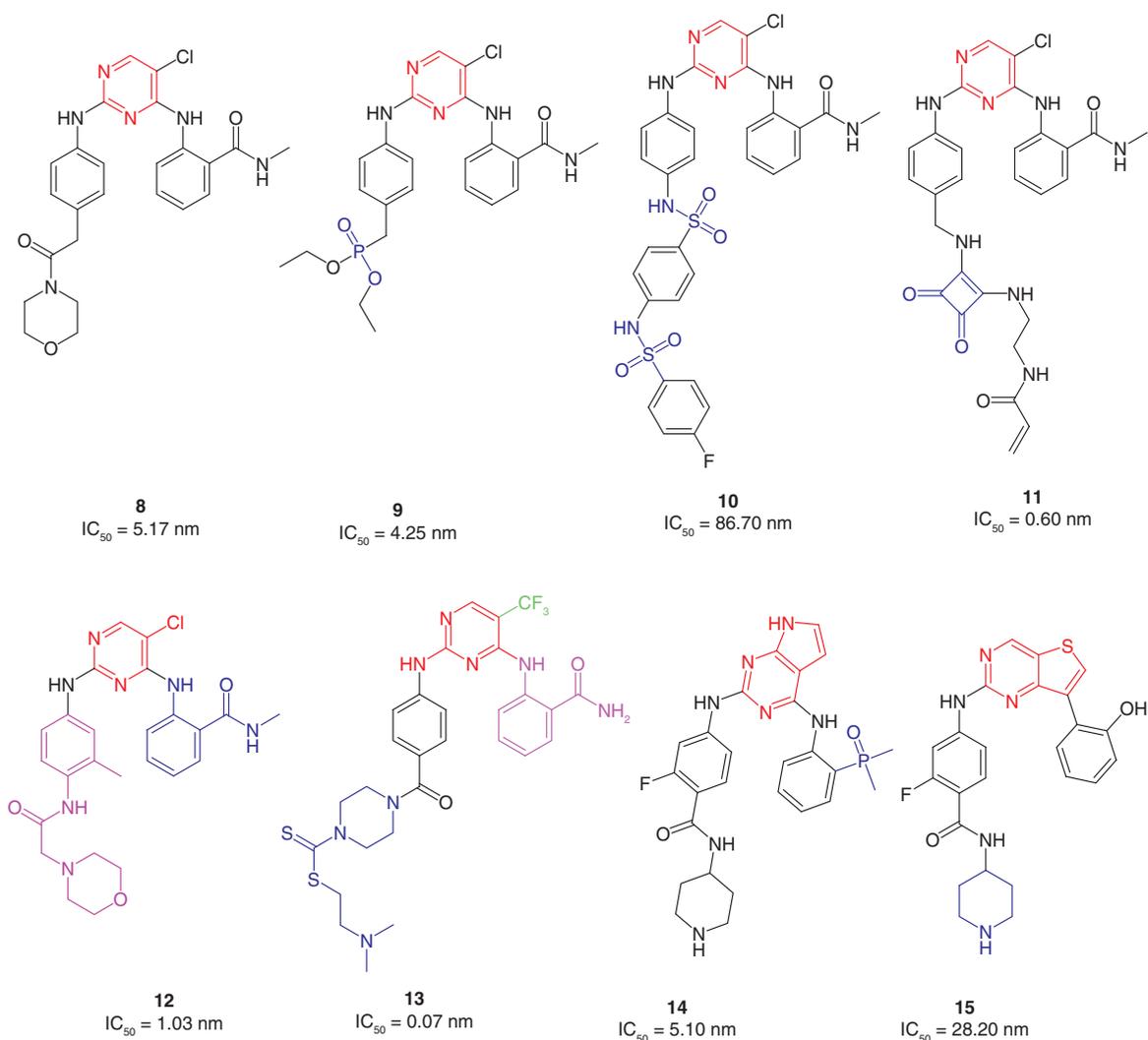


Figure 7. Chemical structure of FAK inhibitors 8–15.

to be less toxic on normal cells than TAE226. Unlike the carbonyl (**8**) and phosphamide (**9**) derivatives, using sulfonamide moiety (**10**) did not retain the anti-FAK activity.

Yen-Pon and colleagues^[125] reported the synthesis of highly potent FAK inhibitors that were able to bind covalently to the ATP binding site of FAK. The prepared derivative **11** exhibited approximately 10-fold better anti-FAK activity than the reference TAE226 (Figure 7). Compound **11** revealed an increased FAK inhibition over 2 h as a time-dependent inhibitory approach, indicating irreversible inhibitory ability^[126]. Interestingly, **11** exhibited potent multiacting inhibitory activity against eight different kinases and showed significant antiproliferative activity against squamous cell carcinoma (SCC) cells with an ED_{50} value of 1.73 μ M (reference VS-4718, $ED_{50} = 1.49$ μ M). In low concentrations, the squaramide derivative **11** inhibited FAK phosphorylation and blocked Tyr397 significantly in SCC cell line, similarly to the reversible FAK inhibitor VS-4718. Washout experiments were performed to test the covalent binding ability of **11**. Unlike VS-4718, the potent inhibitor **11** retained FAK inhibitory activity after washing out.

Recently, Chen and coworkers^[127] reported the synthesis of dual-acting FAK and epidermal growth factor receptor with threonine-to-methionine mutation (EGFR^{T790M}) inhibitors containing *N*-alkylbenzamide-substituted diphenylpyrimidine using fragment-based drug design strategy (FBDD). Upon incorporating the FBDD approach, the researchers merged the two scaffolds of TAE226 and the previously reported EGFR^{T790M} inhibitor *Mor*-DPPY in the same structure (Figure 7)^[128]. MTT assay was used to assess the antiproliferative activity of the alkylbenzamide derivatives against pancreatic cancer (AsPC-1, BxPC-3 and Panc-1), breast cancer (MCF-7/adr) and lung

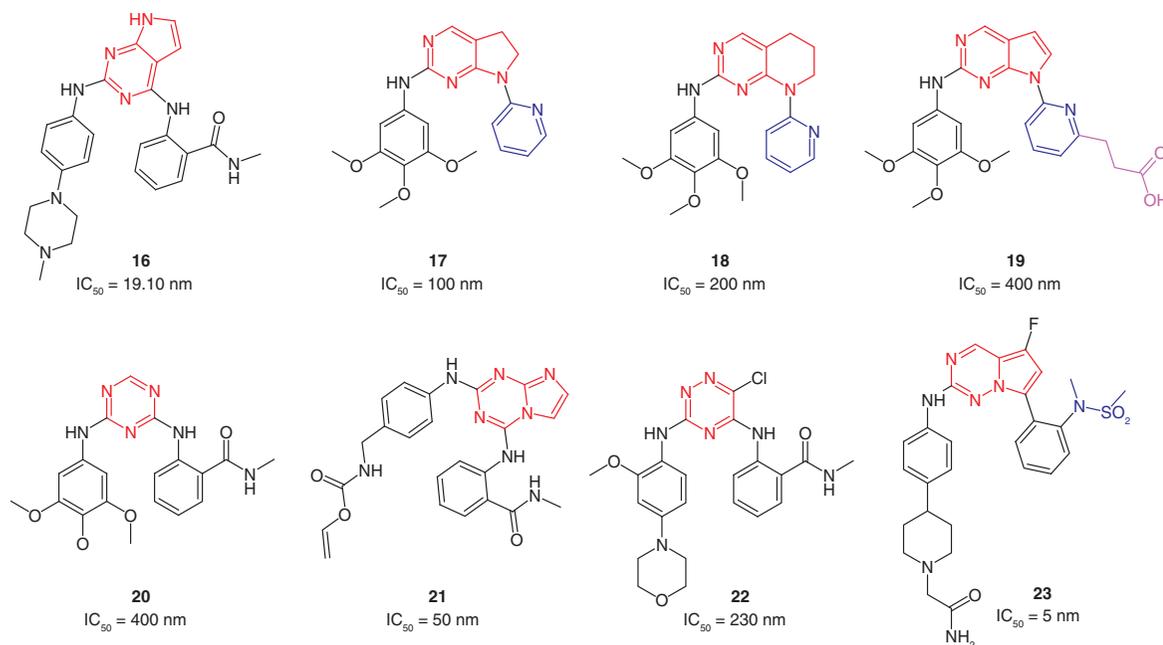


Figure 8. Chemical structure of FAK inhibitors 16–23 with their IC_{50} values.

cancer (H1975) cells. Interestingly, all the prepared derivatives possessed more potent antiproliferative activity than TAE226. Among them, **12** inhibited both FAK and EGFR^{T790M} kinases significantly in nanomolar concentrations and was 6.5-fold more active on FAK kinase than TAE266. The promising results of **12** provoked the researchers to perform further *in vivo* studies on an AsPC-1 cell xenograft mouse model. The results showed 36.6% tumor growth inhibition at 60 mg/kg/q.d. for the oral dosage.

Yin and colleagues [129] synthesized novel 2,4-diarylamino-pyrimidine-based FAK inhibitors bearing dithiocarbamate moiety with potent antiproliferative activity. The research team replaced the morpholine moiety of the reference TAE226 with piperazinyl dithiocarbamate. Surprisingly, **13** showed 88-fold and 11-fold more potent FAK inhibitory activity than TAE226 and defactinib, respectively (Figure 7). The dithiocarbamate derivative exhibited potent anticancer activity on HCT116 colon cancer, PC-3 prostate cancer, U87-MG glioma and MCF-7 breast cancer cells with better activity than both TAE226 and defactinib. Interestingly, **13** was 290-fold and 3000-fold more cytotoxic on HCT116 cells than TAE226 and defactinib, respectively.

Cheng and colleagues [130] designed and synthesized 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives bearing dimethylphosphine oxides as potential FAK inhibitors. Compound **14** showed the best FAK inhibitory activity ($IC_{50} = 5.10$ nM) compared with the synthesized derivatives and was better than TAE226 ($IC_{50} = 6.20$; Figure 7). It also possessed approximately the same anticancer activity on MDA-MB-231 breast cancer and A549 lung cancer cells as TAE226. Moreover, **14** expressed low cytotoxicity on the normal HK2 cells.

The same research group reported a series of 2,7-disubstituted-thieno[3,2-*d*]pyrimidine derivatives [131]. Compound **15** showed better IC_{50} values than TAE226 on U-87MG glioma, A549 lung cancer and MDA-MB-231 breast cancer cell lines (Figure 7). Derivative **15** was tenfold, fourfold and 21-fold more potent than TAE226 on U-87MG, A549 and MDA-MB-231 cells, respectively. This potent anticancer activity was accompanied by a modest ability to inhibit FAK, suggesting that **15** has an off-target mechanism. It was subsequently tested against 25 different kinase enzymes and showed significant activity on five of them (BTK, ALK, CDK2, RET and RET), indicating the multitarget kinase inhibitory activity of this scaffold. Moreover, **15** revealed considerable antimetastatic activity on MDA-MB-231 cell line in a concentration-dependent manner.

Cheng's group [132] also synthesized 7*H*-pyrrolo(2,3-*d*)pyrimidine derivatives using palladium-catalyzed Buchwald cross-coupling. Compound **16** revealed moderate anti-FAK activity ($IC_{50} = 19.10$ nM) compared with TAE226 ($IC_{50} = 7.00$ nM) and showed fourfold, sixfold, and 14-fold better antiproliferative activity than TAE226 in U-87MG, A549 and MDA-MB-231 cells, respectively (Figure 8). It also possessed significant tumor selectivity and showed considerable inhibition on 5 of 9 tested kinases (ABL1, ALK, BTK, FLT3 and KDR) at a concentra-

tion of 1 μM . Interestingly, **16** inhibited the migration of U-87MG, A549 and MDA-MB-231 cells substantially, indicating its significant ability to prevent metastasis.

Choi and colleagues [133] designed several FAK inhibitors containing fused pyrimidines such as saturated and unsaturated scaffolds of both pyrrolopyrimidines and pyridopyrimidines. Compound **17**, bearing the saturated pyrrolopyrimidine, was synthesized through Mitsunobu cyclization and showed the best anti-FAK activity among the prepared compounds ($\text{IC}_{50} = 0.1 \mu\text{M}$; Figure 8). The 5,6,7,8-tetrahydropyrido[2,3d]pyrimidine **18** was found to exhibit a remarkable anti-FAK activity ($\text{IC}_{50} = 0.2 \mu\text{M}$). Based on this study, the same research group performed further docking studies to improve the anti-FAK activity of this scaffold [134]. They suggested that adding an extension to the structure, such as aliphatic carboxylic acid moiety (**19**), would result in extra-interactions inside the FAK active site by forming a salt bridge with Lys454 amino acid. The substitutions in the pyrrolopyrimidine were fixed while the extension was added in the pyridine moiety. Interestingly, the newly prepared compound **19** revealed a substantial anti-FAK activity with an IC_{50} value of 4 nM.

In 2013, Dao and colleagues[42] applied microwave-assisted synthesis for preparing new diarylamino-1,3,5-triazines as FAK inhibitors. The prepared triazine derivatives were tested for their antiproliferative activity on HUVEC human umbilical vein endothelial cells. Compound **20** showed a comparable IC_{50} value to TAE226 on HUVEC cells and revealed a moderate ability to inhibit FAK (Figure 8). The blockage of endothelial-derived FAK activity was confirmed using the FACE™FAK ELISA kit. The results revealed that the autophosphorylation of FAK was inhibited, and the tyrosine 397 phosphorylation of kinase targets was significantly blocked in HUVEC cells in a dose-dependent manner. Compared with TAE226, **20** showed remarkable antiproliferative activity against U-87MG glioblastoma-astrocytoma, HCT-116 colon carcinoma, MDA-MB-231 breast cancer and PC-3 prostate cancer cells [135]. Among 10 tested kinases, **20** showed a significant selectivity on FAK and FGFR2, leading to the shutting down of their signaling pathways. In a dose-dependent manner, **20** significantly decreased cell adhesion and inhibited tube formation leading to alteration of growth, differentiation, migration and proliferation. This effect was accompanied by activation of caspase-3 and induction of apoptosis.

In 2014, Dao's research group explored fusing the imidazole ring with the 1,3,5-triazine moiety. They reported the synthesis of imidazo[1,2-a][1,3,5]triazine derivatives as potential FAK inhibitors [136]. Compound **21** exhibited more potent antiproliferative activity than TAE226 on MDA-MB-231 and PC-3 cells (Figure 8). Moreover, compound **21** substantially inhibited FAK expression and cell adhesion at a low nanomolar level. Compound **21** significantly inhibited cell migration and invasion in U87-MG cells.

In 2017, the same research group synthesized 1,2,4-triazinic FAK inhibitors with strong antiproliferative activity [137]. The researchers added a chlorine atom on position 6 of the triazine ring to simulate the structural features of TAE226 (**1**; Table 1), to facilitate the formation of van der Waals interactions with the gatekeeper amino acid Met499 (**22**; Figure 8). TR-FRET-based kinase assay revealed that **22** possessed anti-FAK activity in submicromolar concentration ($\text{IC}_{50} = 230 \text{ nM}$); however, it was still less potent than TAE226 ($\text{IC}_{50} = 7 \text{ nM}$). Compound **22** showed more potent antiproliferative activity than TAE226 on colon cancer HCT-116 cells, inhibited FAK autophosphorylation and blocked Tyr397 phosphorylation in U-87MG and HCT-116 cells.

Zifcsak and colleagues [138] reported the synthesis of pyrrolo[2,1-f][1,2,4]triazine derivatives as potent inhibitors for FAK and Janus kinase 2 (JAK2). Compound **23** displayed FAK and JAK2 inhibitory activities at the nanomolar level ($\text{IC}_{50} = 5 \text{ nM}$ and 2.5 nM , respectively; Figure 8), and exhibited a remarkable ability to inhibit different kinases; **23** demonstrated more than 90% inhibition on 19 of 53 tested kinases. The phosphorylation of STAT3 decreased substantially (>75%) in a CWR22 tumor xenograft mouse model within 12 h of administration of a single oral dose (55 mg/kg) of **23**. Targeting FAK and JAK2 kinases and STAT3 signaling pathways will inevitably affect tumor cells, but further studies are required to evaluate the obtained antiproliferative activity.

Heinrich and coworkers [139] used FBDD to discover a new FAK inhibitor bearing 1H-Pyrrolo[2,3-b]-Pyridine (**24**), which was able to induce conformational change at the helical DFG motif of the activation loop (Figure 9). N7 and 1H-NH of compound **24** formed two hydrogen bonds with the backbone N-atom and the carbonyl-O of Cys502 in the hinge region, respectively (Figure 9). The cyano group of the 7-azaindole core pointed toward the gatekeeper residue Met499 and made a hydrogen bond with the NH of Asp564 of the DFG motif. The sulfonyl group's oxygen formed a hydrogen bond with the NH of Asp564 amino acid. These two hydrogen bonds may be the reason for the induced conformational change in the DFG motif. Compound **24** exhibited potent FAK inhibitory activity with an IC_{50} value of $0.19 \mu\text{M}$ and showed remarkable antiproliferative activity on HT29 cells ($\text{IC}_{50} = 1.10 \mu\text{M}$).

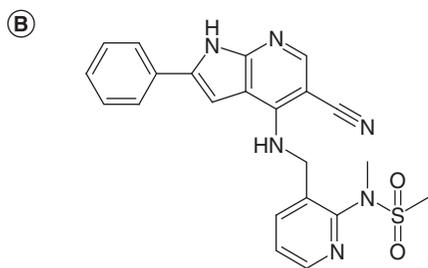


Figure 9. X-ray structure of the ATP binding site of FAK in complex with **24** (yellow), Protein Data Bank code: 4GU6.

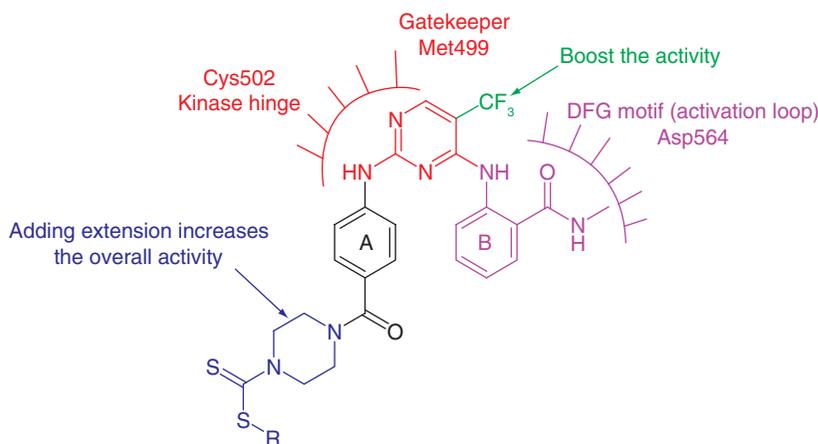


Figure 10. Common features of the six-membered FAK inhibitors.

The investigation of the structure-activity relationship (SAR) of the six-membered FAK inhibitors revealed that most compounds showed potent FAK inhibitory activity. The primary scaffold of this category comprises the presence of diphenyl pyrimidine (Figure 10). Adding various extensions to the first phenyl ring (A) such as the benzocycloheptyl ring (**5**), squaramide moiety (**11**), morpholinoacetamido phenyl (**12**) and dithiocarbamate (**13**) resulted in a dramatic increase in both the anti-FAK and antiproliferative activity. These extensions could allow the formation of extra-interactions with Glu506, Ile428, Gly429, Asn556 and Leu567 amino acids inside the ATP binding site of FAK [86]. On the other hand, ring B contained *N*-methylbenzamide moiety in most derivatives and did not experience modifications except for some successful trials using pyridine, pyrazine rings or adding methylsulfonyl amine substitution. Replacing the chloro with the strong electron-withdrawing trifluoromethyl group in C-5 of the pyrimidine moiety substantially boosted the activity (compounds **1**, **3**, **4**, **6** and **13**). Using fused pyrimidines, triazines or fused triazines instead of the pyrimidine ring did not enhance the FAK inhibitory activity, whereas incorporating the pyridine moiety resulted in a remarkable increase in potency (**2**). Computational methods such as FBDD succeeded in retaining and enhancing anti-FAK and antiproliferative activity (compound **12**). Despite promising activity, most of the prepared six-membered inhibitors were not selective on FAK; targeting covalent bond formation inside the ATP binding site of FAK, such as the squaramide derivative **11**, could improve anti-FAK activity and selectivity.

Five-membered heterocyclic FAK inhibitors

Sun and colleagues [140] reported the synthesis of 1,3,4-thiadiazole derivatives bearing 1,4-benzodioxan as potential FAK inhibitors. The most potent derivative (**25**, Figure 11) showed FAK inhibitory activity with an IC_{50} value of 10.79 μ M compared with staurosporine (IC_{50} = 11.32 μ M). Moreover, it inhibited hepatocellular carcinoma HepG2 cells substantially and was more potent than 5-fluorouracil and staurosporine. Despite the enzymatic inhibitory activity, **25** did not retain the antiproliferative activity on Hela (cervical cancer), SW1116 (colorectal cancer) or BGC823 (gastric cancer) cell lines. western blot analysis showed that **25** significantly repressed FAK phosphorylation at a concentration of 12.5 μ M.

The same research group reported the synthesis of a series of 1,3,4-oxadiazole derivatives [141]. The target derivatives showed promising FAK inhibitory activity in low micromolar concentrations, in which **26** was the

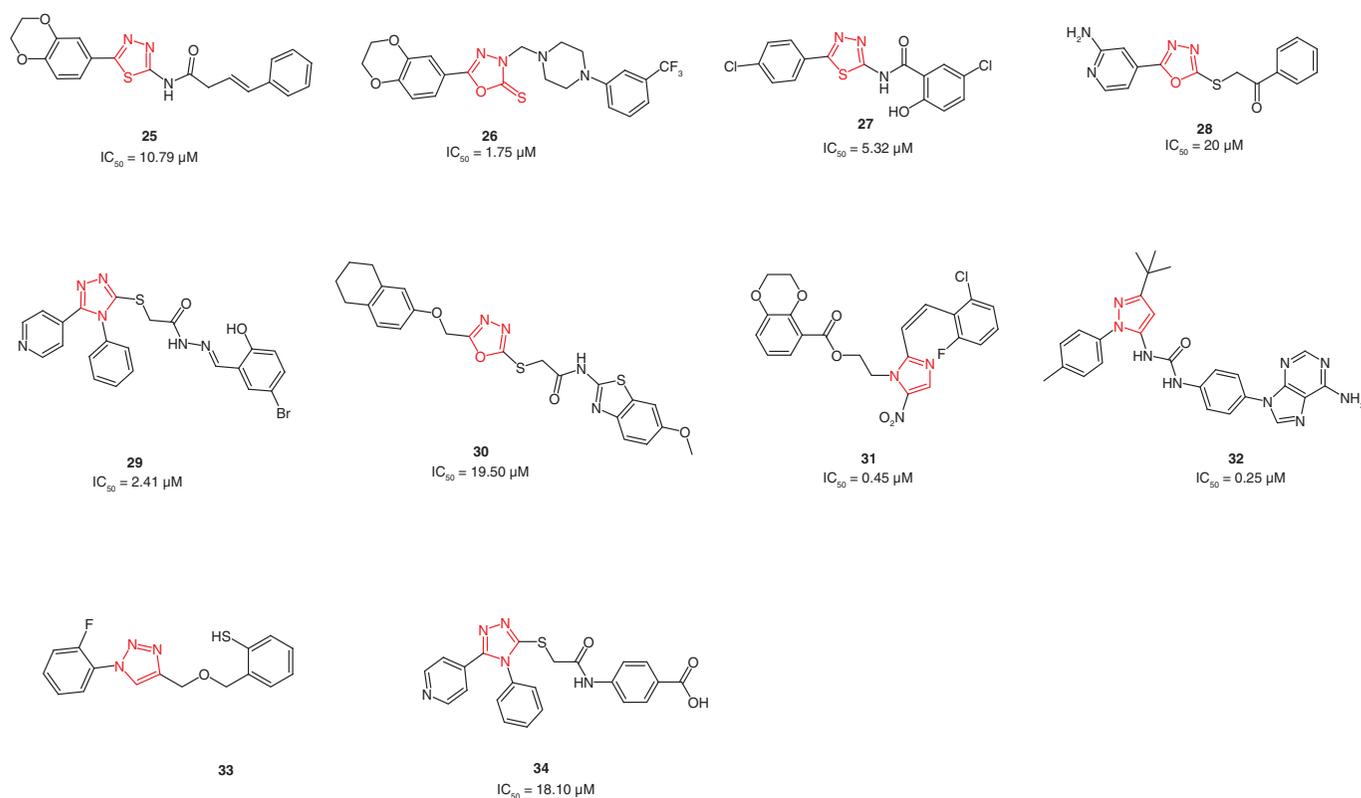


Figure 11. Chemical structure and biological activity of FAK inhibitors 25–34.

most active (IC₅₀ = 0.78 μM; Figure 11). Derivative **26** retained antiproliferative activity in HepG2 cells and was twofold more active than 5-fluorouracil. However, **26** showed mild potency on Hela, SW1116 and BGC823 cell lines. Replacing the butanamide (**25**) with piperazine moiety, along with the presence of the trifluoromethyl group (**26**), enhanced the FAK inhibitory activity.

Zhu and coworkers [142] reported the synthesis of 1,3,4-thiadiazol-2-amides as potent FAK inhibitors. The obtained antiproliferative activity of the prepared derivatives revealed that compound **27** was able to inhibit the growth of MCF-7 breast cancer and B16-F10 melanoma cell lines better than staurosporine (Figure 11); **27** showed twofold better anti-FAK activity than staurosporine. Western blot analysis emphasized that **27** exerted its activity through FAK inhibition. Compound **27** is considered an excellent example of retaining both antiproliferative and anti-FAK activity.

The same research group reported the synthesis of a series 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanones with moderate antiproliferative and anti-FAK activity [143]. The group incorporated the oxadiazole ring instead of the thiadiazole as an approach for bioisosteric replacement [142]. Unfortunately, most of the synthesized derivatives did not show potent activity, excluding **28**, which showed superior activity on MCF-7 breast cancer and A431 epidermoid carcinoma cells (Figure 11). Compound **28** showed an IC₅₀ value sevenfold better than the reference staurosporine in A431 cells. Favorably, **28** was 1.5-fold more potent than staurosporine on FAK enzyme inhibition.

Qiu and coworkers synthesized novel pyridinyl-1,2,4-triazole-based FAK inhibitors possessing acetohydrazide moiety [144]. Compound **29** showed the best potency among the prepared compounds and retained both antiproliferative and anti-FAK activities (Figure 11). The pyridinyl-1,2,4-triazole **29** possessed potent FAK inhibitory activity with an IC₅₀ value of 2.41 μM, whereas staurosporine showed an IC₅₀ value of 3.12 μM. Compound **29** inhibited MCF-7, HCT116 and HepG2 cells 2.3-fold, 6.6-fold and twofold better than staurosporine, respectively. The western blot analyses confirmed that the potent antiproliferative activity of this series is attributed to the significant inhibition of FAK.

Recently, Altuntop and colleagues [145] reported dual-acting Akt and FAK inhibitors bearing 1,3,4-oxadiazole moiety. Using MTT assay, compound **30** showed more potent antiproliferative activity than cisplatin and GSK690693 on A549 lung adenocarcinoma, C6 rat glioma and NIH/3T3 mouse embryonic fibroblast cells (Figure 11). Com-

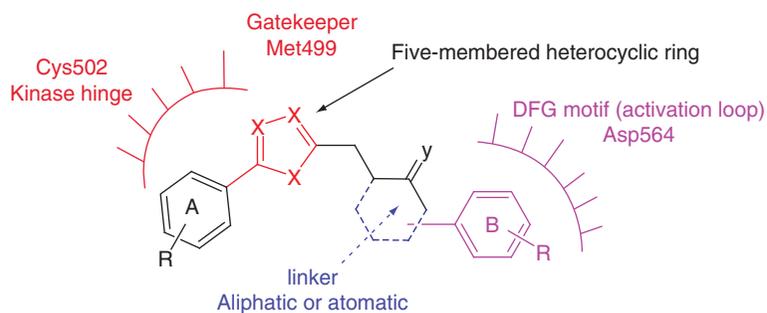


Figure 12. Common features of the five-membered FAK inhibitors.

compound **30** showed potent FAK inhibitory activity with an IC_{50} value of $19.50 \mu\text{M}$, and possessed better Akt inhibitory activity than cisplatin and GSK690693.

In 2014, a series of 2-styryl-5-nitroimidazole derivatives bearing 1,4-benzodioxan moiety was synthesized as potential FAK inhibitors [45]. Compound **31** showed the same ability to inhibit A549 lung cancer and hela cervical cancer cells as staurosporine ($IC_{50} = 3.11$ and $2.54 \mu\text{M}$, respectively; Figure 11). Compared with staurosporine ($IC_{50} = 0.50 \mu\text{M}$), **31** exhibited potent FAK inhibitory activity ($IC_{50} = 0.45 \mu\text{M}$).

Grädler and colleagues [146] used fragment-based lead discovery (FBLD) to develop novel FAK inhibitors. The team screened 1920 fragments against the kinase domain of FAK using surface plasmon resonance (SPR) [147]. Protein crystallography revealed the binding mode of only 41 fragment hits in the ATP binding site of FAK. The output of this study revealed a novel pyrazole-containing compound (**32**) that inhibits FAK in low micromolar potency, $IC_{50} = 0.266 \mu\text{M}$; $K_{D,ss} = 0.111 \mu\text{M}$ ($K_{D,ss}$ is the steady state dissociation constant that is determined by plotting the binding levels at the end of the association phase against the concentration; Figure 11). Compound **32** inhibited FAK significant phosphorylation at Tyr397 in HT-29 human colorectal adenocarcinoma cells ($IC_{50} = 0.75 \mu\text{M}$). Surprisingly, **32** showed a significant multiacting kinase inhibitory activity and inhibited 14 out of 48 tested kinases.

Jia and colleagues [148] prepared thiophenol-formaldehyde-triazoles as potential antiovarian cancer derivatives with potent anti-FAK activity. After 48 h treatment, compound **33** revealed a marked lessening in FAK activation in CAOV3 ovarian cancer cells at a concentration of $0.5 \mu\text{M}$ (Figure 11). Compound **33** considerably inhibited cell viability of ES-2, CAOV3 and CAOV4 ovarian cancer cells. This compound significantly decreased CAOV3 migration in a concentration-dependent manner and decreased the infiltration of macrophages in different metastatic lesions from the intestinal, liver and pulmonary tissues.

Recently, Mustafa and colleagues [149] reported 5-pyridinyl-1,2,4-triazoles containing acetamido carboxylic acids as potent FAK inhibitors. The prepared derivative **34** showed strong antiproliferative activity in hepatocellular carcinoma HepG2 ($IC_{50} = 3.78 \mu\text{M}$) and Hep3B ($IC_{50} = 4.83 \mu\text{M}$) cells (Figure 11). Compound **34** showed better anti-FAK inhibitory activity ($IC_{50} = 18.10 \text{ nM}$) than the reference GSK2256098 ($IC_{50} = 22.14 \text{ nM}$). Immunoblotting analysis in HepG2 cells revealed a substantial FAK phosphorylation repression. The obtained FAK inhibition decreased the phosphorylation in PI3K, Akt, JNK and STAT3.

The five-membered nitrogen-containing FAK inhibitors are considered an important category because this scaffold is amenable to modifications that allow the molecular hybridization approach. Most prepared derivatives retained anti-FAK and antiproliferative activities, such as compounds **27**, **28**, **29**, **32** and **34**. SAR analysis revealed that the presence of the diphenyl substitution (ring A and ring B) on the five-membered heterocyclic ring is critical for activity (Figure 12). Molecular docking of most compounds revealed that the heterocyclic ring and ring A, (such as diphenyl dioxine, substituted phenyl or pyridinyl) bind to Cys502 of the kinase hinge, while ring B attaches to the heterocyclic ring through an aliphatic or aromatic linker that allows interaction with Asp564 of the DFG motif (Figure 12). The five-membered heterocyclic ring points to the gatekeeper residue Met499. The bioisosteric replacements such as 1,3,4-oxadiazole, 1,3,4-thiadiazole, different triazoles, imidazole and pyrazole were successful in most attempts.

Allosteric FAK inhibitors

Another novel method of inhibiting FAK is to target the allosteric site within the kinase domain, which is distant from the ATP active site (Figure 13). Allosteric inhibition is a highly selective strategy for targeting various kinases;

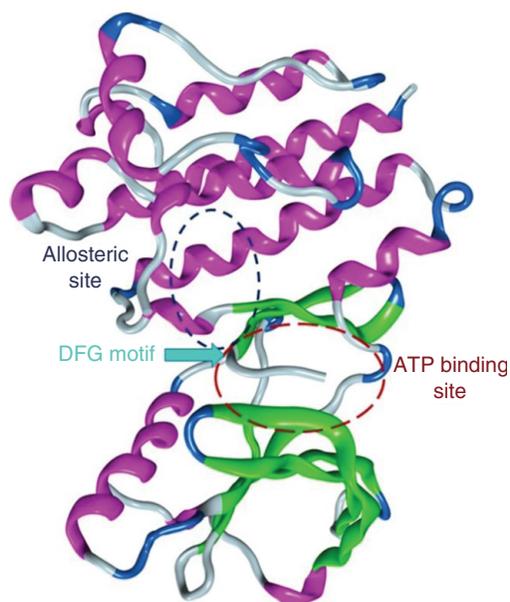


Figure 13. The FAK illustrates the ATP active binding site and the allosteric site.

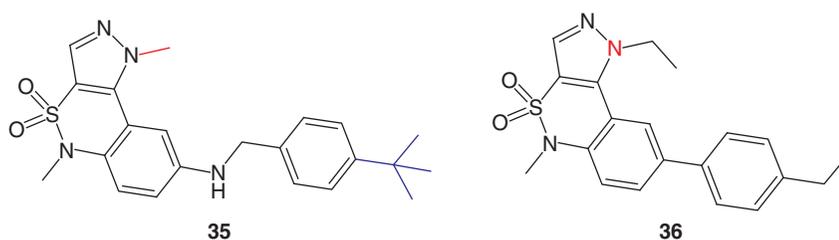


Figure 14. Chemical structure of the allosteric FAK inhibitors **35** and **36**.

for example, PD184352 and Akt-I-1 are allosteric inhibitors that target mitogen-activated protein kinase (MEK) and Akt, respectively [150,151].

Tomita and colleagues [152] used high-throughput screening to report 1,5-dimethyl-1,5-dihydropyrazolo[4,3-c][2,1]benzothiazine (**35**) as a potent allosteric inhibitor of FAK (Figure 14). Compound **35** showed considerable selectivity on FAK enzyme upon testing eight different kinases, with an IC_{50} value of 0.64 μ M. This effect was obtained due to the significant drop in FAK autophosphorylation level in PC-3M-luc prostate cancer cells. Compound **35** suited the allosteric FAK active site perfectly and formed several hydrogen-bonding interactions with Gly563, Asp604, Met607, Phe608, Cys611 and Arg550 (Figure 15). The methyl group on the pyrazole moiety was also subjected to the hydrophilic space, and the tertiary butyl group occupied the hydrophobic pocket. Arg550 made a cation- π interaction with the pyrazole ring.

Miki and coworkers reported a potent inhibitor (**36**) with the same scaffold as **35**; however, the 1,5-dihydropyrazolo[4,3-c][2,1]benzothiazine moiety was directly attached to the 4-ethylphenyl group at position 8 (Figure 14) [153]. The derivative **36** bound to the FAK allosteric site with a slow dissociation rate on unphosphorylated FAK, leading to ATP noncompetitive FAK inhibition. Compound **36** was highly selective toward FAK. However, eliminating the ethyl group from N^1 of the pyrazole ring resulted in nonselective FAK inhibitory activity, giving the impression that pyrazole N substitution is necessary for achieving FAK selectivity and allosteric binding. Targeting the allosteric site of FAK is a promising approach because the obtained inhibitors revealed strong FAK selectivity. However, additional research should be conducted to further optimize the structures and increase the anti-FAK potency.

Targeting FAT & FERM domains as potential inhibitors of FAK

Several studies reported the similarity of different kinases to the ATP binding site of FAK, which inevitably leads to off-target effects due to the lack of selectivity [154–156]. FAT domain contains Tyr925 as a key phosphorylation site

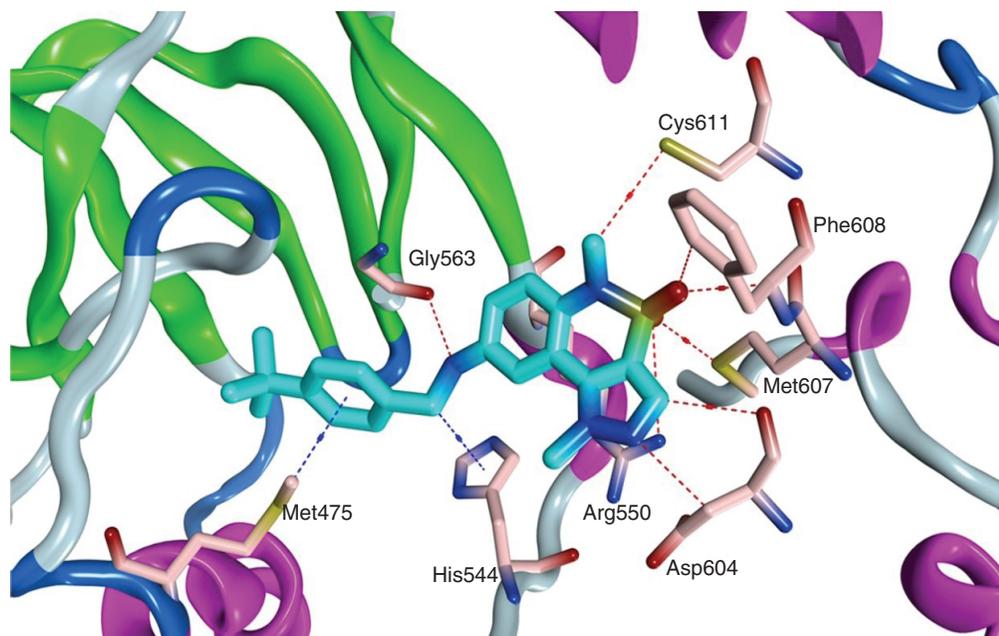


Figure 15. Cocrystal structure of 35 within the allosteric binding site of FAK, Protein Data Bank code: 414F.

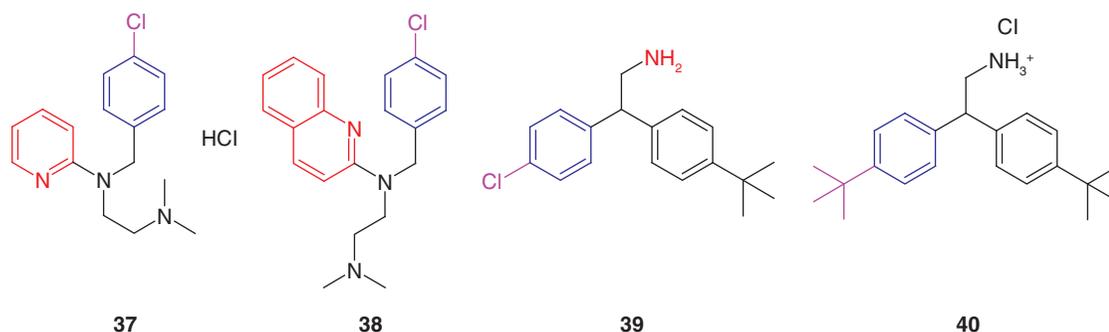


Figure 16. Chemical structure the FAK-FAT domain inhibitors 37–40.

that, upon its phosphorylation, metastasis and invasion of tumors, is significantly promoted [157]. Likewise, Tyr397 autophosphorylation leads to FAK activation [158,159]. Targeting these phosphorylation sites (Tyr925 and Tyr397) leads to FAK inhibition. Therefore, several trials have targeted FAT or FERM domains of FAK [160,161].

FAK-FAT domain inhibitors

Cance and coworkers [162] discovered a small molecule (chloropyramine hydrochloride, **37**) as a target for FAK and VEGFR-3 binding sites with the help of the computational docking techniques (Figure 16). The histamine receptor antagonist **37** was selected out of 140,000 molecules to target the FAT domain of the FAK protein. Compound **37** induced abrogation to the phosphorylation of VEGFR-3 and FAK. This potent antikinase activity resulted in considerable antiproliferative activity on BT474 and T47D breast cancer and C8161 melanoma cells, with IC_{50} values lower than 5 μ M. Compound **37** revealed a dramatic decrease in the FAK and VEGFR-3 colocalization in the cytoplasm of BT474 and MCF7-VEGFR3 cells. This is considered a novel method for targeting the protein–protein interactions of FAK and VEGFR-3.

The same research group synthesized several analogs of **37** to seek better activity [163]. Their efforts revealed compound **38**, which exhibited significant antiproliferative activity in MiaPaCa-2-luc and Panc-1-luc pancreatic cancer and MCF7-VEGFR-3 cell lines (Figure 16). Compound **38** considerably inhibited FAK-VEGFR-3 interaction better than **36** in MiaPaCa-2-luc cells. Compound **38** was tested against 10 different kinases; however, it

did not show any significant activity, implying that **38** targets a specific FAK-VEGFR-3 interaction site. Molecular docking revealed a remarkable ability of **38** to occupy the FAK-FAT pocket with several key hydrophobic and electrostatic interactions; the quinoline ring made a cation- π interaction with Lys1032 amino acid along with a π -stacking interaction between Tyr925 and the *p*-chlorobenzyl group.

Recently, Kandil and colleagues^[164] employed structure-based drug design and virtual screening to filter a library of 210,000 compounds against the FAK-FAT domain to identify 25 virtual hit compounds screened in the invasive MDA-MB-231 cell line. Compound **39** revealed significant antiproliferative and antimigratory properties in low micromolar concentration (Figure 16). These results exceeded those of chloropyramine, which may be attributed to the presence of the amino group that makes a salt bridge with the carboxyl group of Asp1036, and to the formation of two hydrogen bonds with the carbonyl group of Pro911 and the hydroxy group of Ser910 amino acid [162]. Compound **39** was considered a starting point that led to studying the impact of adding different substituents to the diarylamine scaffold; the prepared derivatives possessed better antiproliferative activity than chloropyramine in T47D, BT474 and MDA-MB-231 breast cancer and MIA-Paca2 pancreatic cancer cell lines. The substitution variation resulted in a slight increase in the antiproliferative activity for compound **40** over compound **39** in most tested cells (Figure 16).

Interestingly, the study of the FAT-domain targeting inhibitors revealed that all the prepared compounds share the same scaffold, and only structural optimization was required. Replacing the chloro group of compounds **37–39** with trifluoromethoxy or tertiary butyl groups increased the antiproliferative activity (**40**). On the contrary, adding a methoxy group or aliphatic side chains in the same position led to a drastic decrease in potency. Adding quinoline moiety (**38**) instead of the pyridine (**37**), boosted the activity.

FAK-FERM domain inhibitors

Golubovskaya and colleagues^[165] used a structure-based *in silico* molecular docking approach to target the Tyr397 phosphorylation site of FAK. A total of 140,000 small molecules were docked into the N-terminal domain of FAK kinase domain (PDB code: 2AL6) in 100 different orientations [17]. The top 35 molecules found to best fit into the Tyr397 phosphorylation site in the FERM domain with the best energy scores, were tested on six different cancer cells. Only one compound (1,2,4,5-benzenetetraamine tetrahydrochloride, **41**, **Y15**) possessed promising antiproliferative activity in all the tested cells and was comparable to the reference TAE226 (Figure 17). Compound **41** inhibited BT474 cancer cell viability and FAK phosphorylation in a dose-dependent manner. Western blot analysis revealed that **41** specifically inhibited Tyr397 FAK phosphorylation and caused less cellular detachment than TAE226 in BT474 cells, indicating a significant ability of **41** to prevent metastasis. The *in vivo* studies performed in nude mice revealed that **41** blocked tumor growth and reduced tumor weight in BT474 cells at a dose of 30 mg/kg. Western blotting and immunohistochemical staining of tumors showed decreased Tyr397-FAK phosphorylation levels in **41**-treated mice. The docking pose of **41** revealed the formation of four hydrogen bonds with Tyr397, Glu399, Arg57 and Thr394 amino acids, and was in higher proximity to the essential amino acid Tyr397.

Compound **42**, also known as **Y11**, was identified by the same research group, as a potent FAK inhibitor that targets Tyr397 site (Figure 17) [160]. The azoniatricyclodecane derivative **42** blocked the autophosphorylation of FAK effectively and significantly lessened Tyr397 phosphorylation in BT474 (breast cancer) and SW620 (colon cancer) cells. The octet binding assay confirmed the ability of **42** to bind directly to the FAK-N-terminal domain. Interestingly, **42** prevented colony formation in BT474 and SW620 cancer cells. The *in vivo* studies revealed that **42** (125 mg/kg) decreased tumor growth and weights in SW620 xenograft mice tumor. Additionally, it decreased Tyr397-FAK phosphorylation, and increased caspase-3 staining in tumor xenografts.

Thiyagarajan and colleagues^[166] conducted docking studies to discover the natural compound 16-hydroxycloeroda-3,13-dien-16,15-olide (HSD, **43**). MTT assay revealed that **43** significantly inhibited N18 neuroblastoma and C6 glioma cells in 10 μ M concentration (Figure 17). Compound **43** increased p53 expression and downregulated B-cell lymphoma 2 (Bcl-2 gene family comprises a group of related genes that regulate apoptosis) resulting in significant apoptosis induction [167]. Western blotting and immunofluorescence assays showed that **43** decreased the Tyr397-FAK expression level in C6 and N18 cells.

The same research group applied virtual screening to discover nifedipine as potent FAK and 70-kDa ribosomal S6 kinase 1 (S6K1) inhibitor [168]. Neferine (**44**) was able to bind perfectly to FAK-FERM domain and decreased phosphorylated FAK and S6K1 (Figure 17). Compound **44** showed better antiproliferative activity than cisplatin in C6

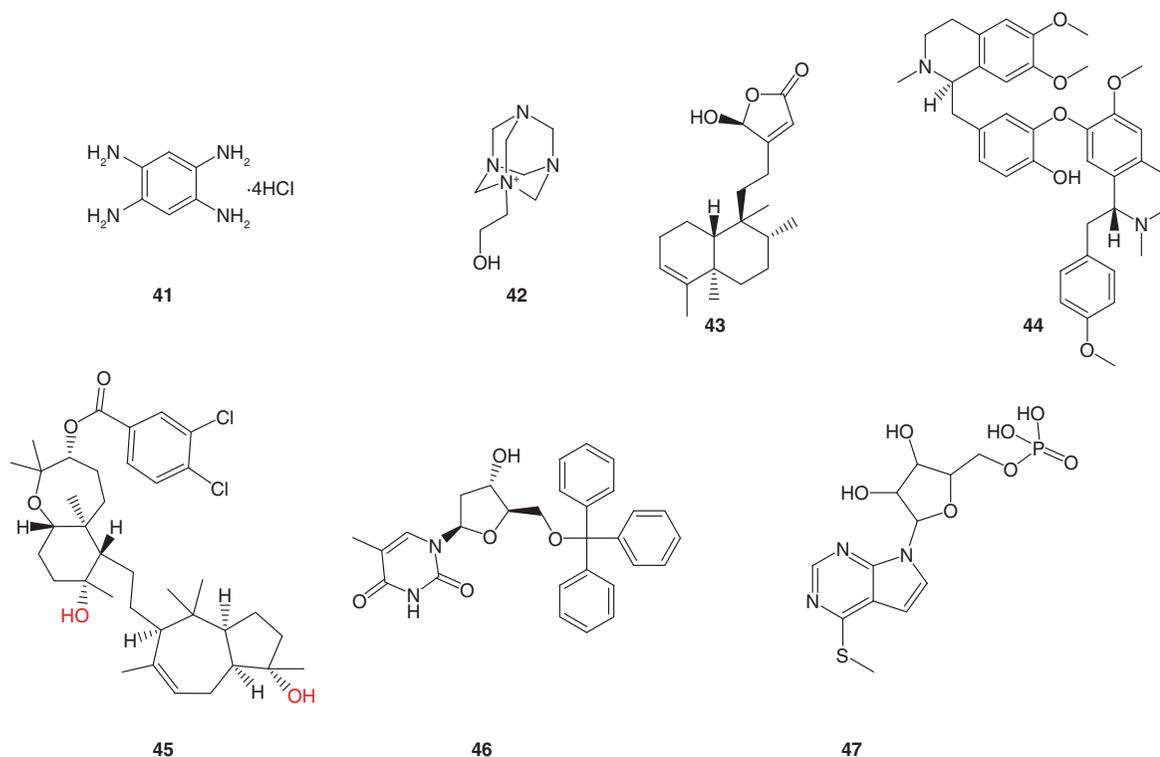


Figure 17. Chemical structure FAK-FERM domain inhibitors 41–47.

cells. Additional studies performed on neferine revealed that the dual FAK/S6K1 inhibitory activity of neferine results in potent anticancer activity in human neuroblastoma [169].

El Sayed and coworkers [170] studied a derivative of the natural compound siphonolol A called siphonolol A-4-O-3',4'-dichlorobenzoate (SAP, **45**) that was found to inhibit breast cancer cells with promising IC_{50} values, including MDA-MB-231, MCF-7, BT474 and T47D (Figure 17). Western blot analysis revealed that **45** resulted in significant FAK phosphorylation inhibition at Tyr397 site in all tested cancer cells. Moreover, **45** inhibited the phosphorylation of breast tumor kinase (a nonreceptor tyrosine kinase overexpressed in most invasive human breast tumors, with a fundamental role in promoting cancer proliferation, invasion and migration) [171,172]. *In vivo* experiments revealed a significant reduction in tumor volume, weight and growth (76% reduction) in a dose of 10 mg/kg. Docking **45** into the x-ray crystal structure of the FAK-FERM domain revealed the formation of two hydrogen bonds between the hydroxyl groups and Tyr394 and Glu399 amino acids, while the bicyclic decane ring formed a hydrophobic interaction with Tyr397's phenyl group.

Golubovskaya and colleagues [173] performed *in silico* molecular docking for more than 200,000 small molecules obtained from the NCI database to target FAK-MDM-2 interactions. The study revealed 24 optimal compounds that can target that complex. These compounds were tested for their antiproliferative activity in breast, colon, melanoma and pancreatic cancers using MTT assay. Among them, **46** had the best inhibitory activity in most tested cells (Figure 17). Compound **46** significantly inhibited the viability of BT474 breast cancer cells and increased cell detachment and apoptotic induction. Western blot analysis revealed a profound ability of **46** to decrease FAK and Tyr397 FAK, increase Mdm-2 apoptotic level, and activate P53 and caspase-8 in BT474 cells. At 100 μ M, **46** decreased FAK and Mdm-2 complex formation substantially in HCT116 colon cancer and BT474 cells. *In vivo* experiments performed in a xenograft mouse model revealed a significant ability of **46** to decrease breast and colon tumor growths.

Deniz and colleagues [174] used INT2–31 (**47**) to target protein–protein interactions of FAK with cMET and IGF-1R (Figure 17). Compound **47** decreased the phosphorylation of Tyr397 in both Panc-1 and MiaPaca-2 pancreatic cancer cells and possessed potent anticancer activity in different types of cancer (breast, melanoma, esophageal and pancreatic cancer). This compound disrupted the binding of IGF-1R and cMET proteins and blocked the interaction of FAK with IGF-1R and cMET. *In vivo* studies using a xenograft mouse model revealed

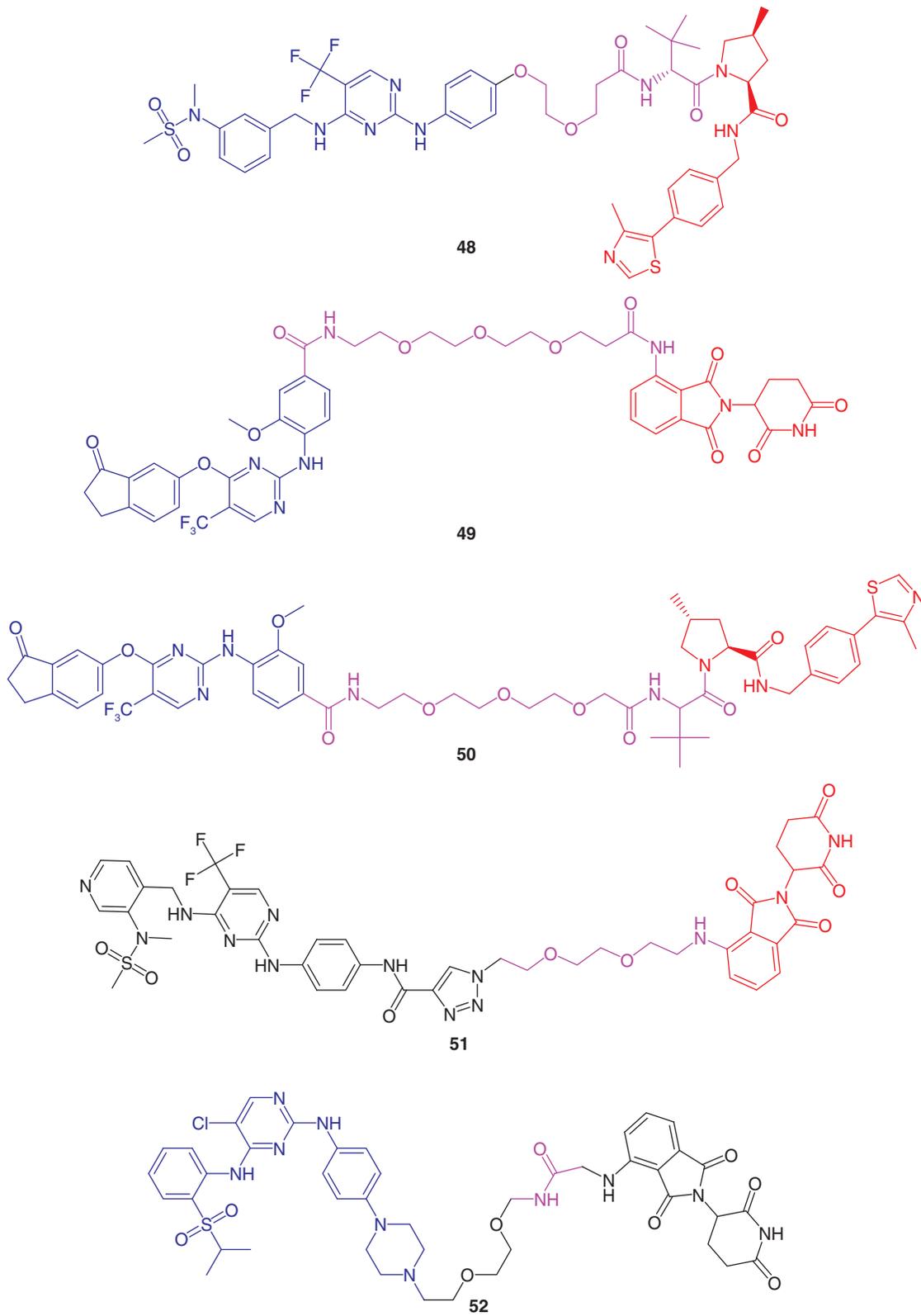


Figure 19. Chemical structures of FAK proteolysis targeting chimeras 48–52.

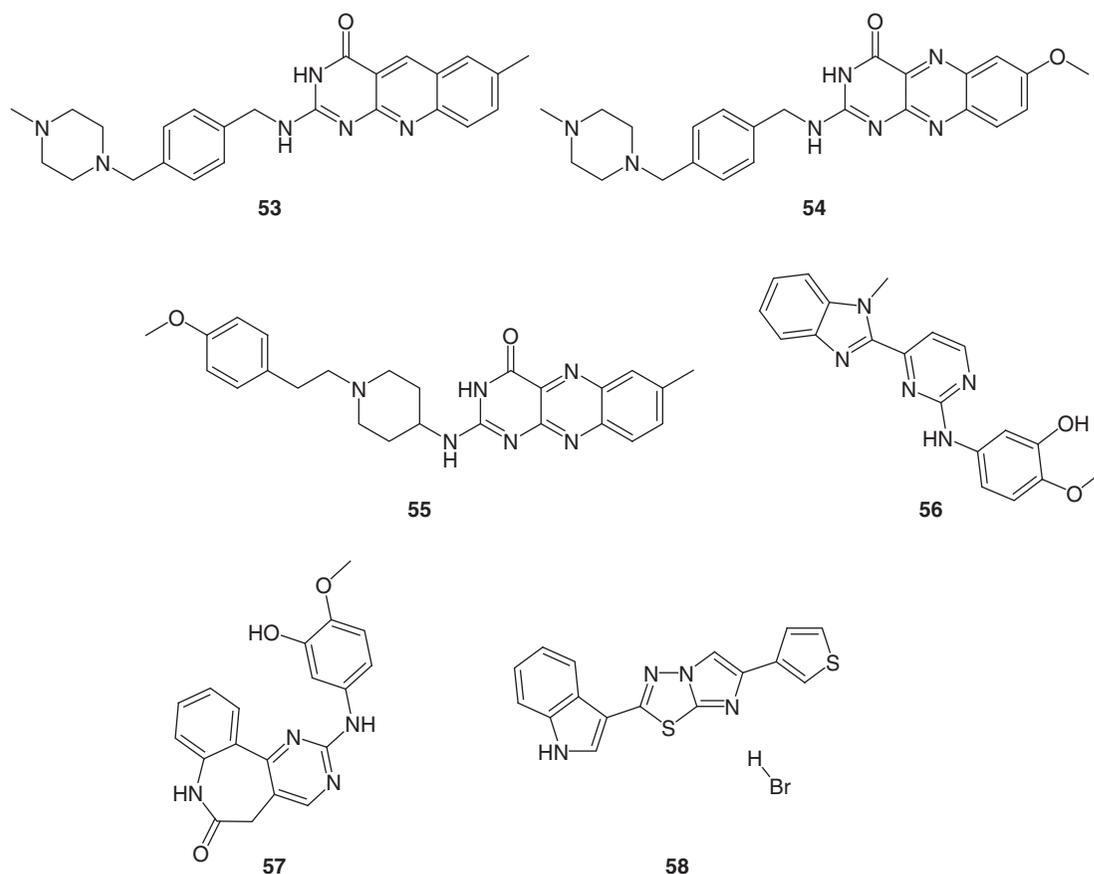


Figure 20. Chemical structure and biological activity of miscellaneous FAK inhibitors 53–58.

Miscellaneous FAK inhibitors

Some FAK kinase inhibitors do not meet the typical modular structural form of the five- and six-membered FAK inhibitors. The selective FAK inhibitors in this miscellaneous category and are not easily classified in the manner outlined in this review, and are presented separately here. Ali and colleagues [183] reported the synthesis of alloxazine and 5-deazaalloxazine derivatives through nitrosative cyclization and Vilsmeier-Haack cyclization, respectively. Compounds **53**, **54** and **55** revealed multiacting kinase inhibitory activity and exhibited significant potency on most tested cells, such as MCF7 breast cancer, A2780 ovarian cancer, HCT116 colon cancer and WM793B melanoma cell lines (Figure 20). Compound **53** showed approximately 13-fold and 32-fold more potent antiproliferative activity than Lapatinib on MCF7 and A2780 cells, respectively. Interestingly, **54** showed a dramatic increase in antiproliferative activity; it was 178-fold more potent than imatinib in both MCF7 and A2780 cells, while on HCT116, **54** was 393-times more active than imatinib. The synthesized compounds were tested for their ability to inhibit 20 different kinases. Compound **53** inhibited FAK (48%) and ABL1 (33%), whereas both **54** and **55** showed 34% inhibition of CDK. Compounds **54** and **55** inhibited FAK by 10% and 21%, respectively. Using a fused heterocyclic ring, such as alloxazine, may be a promising approach in inhibiting various kinases.

Determann and colleagues [184] prepared a series of 2-anilino-4-(benzimidazol-2-yl)pyrimidine derivatives. Among the synthesized compounds, **56** showed considerable anti-FAK ($IC_{50} = 3.40 \mu M$) and antiproliferative activity (Figure 20). Using docking studies, the research team determined that their previously reported compound (**57**) could bind perfectly in the ATP active site in protein kinases (Figure 20) [185]. Compound **57** showed potent FAK inhibitory activity ($IC_{50} = 0.64 \mu M$) compared with sunitinib ($IC_{50} = 1.60 \mu M$). Compounds **56** and **57** inhibited three kinases efficiently (PLK1, VEGF-R2 and Aurora B). Compound **57** also showed better VEGF-R2 inhibition than sorafenib and equipotent activity to sunitinib. The antiproliferative activity of **56** and **57** was assessed on ten different cancer cells, and the best activity was obtained on MCF7, CAKI-1, MDA-MB-435, OVCAR-3 and SW620 cells.

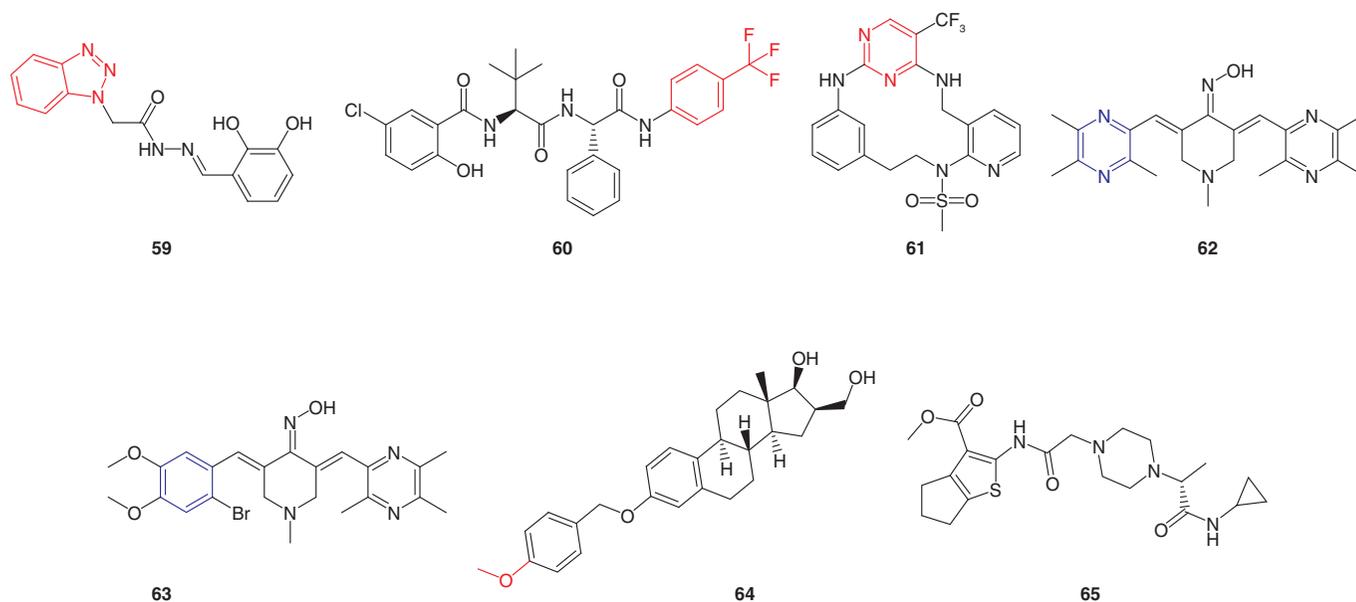


Figure 21. Chemical structures of miscellaneous FAK inhibitors 59–65.

Using Vilsmeier conditions, Diana and colleagues^[186] reported a series of hybrid molecules containing imidazo[2,1-*b*][1,3,4]thiadiazole and indole moieties with potent FAK inhibitory activity. Compound **58** showed potent antiproliferative activity in leukemia K-562, colon cancer HT29, SW-620, and melanoma MDA-MB-435 cells (Figure 20). The sulforhodamine-B assay revealed that **58** possessed significant antiproliferative activity in PDAC cells; SUIT-2, Capan-1 and Panc-1. Compound **58** showed a significant reduction of PDAC-3 tumor sphere volume after 8 days of treatment in a dose of 8.5 μM and induced a considerable reduction in cell migration rate in SUIT-2 and Panc-1R cells. Western blot analysis revealed that **58** inhibited the phosphorylation of FAK significantly.

In 2018, Kassab and Hassan prepared hybrids of benzotriazole and *N*-acrylylhydrazone moieties as potent FAK inhibitors [43]. Compound **59** was approximately equipotent to the reference GSK-2256098 ($\text{IC}_{50} = 44.60 \text{ nM}$) and inhibited the tyr397 phosphorylation in a nanomolar concentration ($\text{IC}_{50} = 32.72 \text{ nM}$; Figure 21). Favorably, **59** revealed more than 70% growth inhibition on CCRF-CEM, HL-60 leukemia and OVCAR-3 ovarian cancer cells. Based on the obtained NCI results, OVCAR-3 and HL-60 cells were chosen for further analysis using MTT assay; **59** showed 10- and 18-times more potent antiproliferative activity on OVCAR-3 and HL-60 cells than doxorubicin, respectively.

Additionally, the peptide drug discovery approach was investigated by Krystof and his research team by developing noncanonical modified leucine dipeptides [187]. At early time points, dipeptide **60** showed significant dephosphorylation of FAK, paxillin, and p130Cas at Tyr397, Tyr118 and Tyr410, respectively (Figure 21). This effect was accompanied by a gradual decrease in HCT-116 proliferating cells. This study is an excellent example of the use of dipeptides to inhibit FAK enzyme. Heck-mediated macrocyclization was implemented for preparing PF-562271 analogs by Farand and his research team [188]. The Macrocycle **60** provided enhanced selectivity on FAK and Pyk2 over the noncyclized form by 1.2-fold and 19-fold, respectively (Figure 21).

Bukhari and coworkers [189] synthesized a series of piperidin-4-one oxime derivatives bearing ligustrazine via Claisen-Schmidt condensation reaction. The ligustrazine derivatives **62** and **63** possessed the best activity among the synthesized compounds and showed more potent FAK inhibitory activity than erlotinib with IC_{50} values of 2.90 μM and 1.90 μM , respectively (Figure 21). Compounds **62** and **63** exhibited strong antiproliferative activity in nanomolar concentrations against A549 lung cancer, PC-3 prostate cancer, MCF7 breast cancer, PaCa-2 pancreatic cancer and HT29 colon cancer cells. The main structural difference between the two piperidin-4-one oxime derivatives is that the pyrazine moiety of **62** was replaced by a substituted phenyl ring in **63**. Compounds **62** and **63** showed a wide variety of mechanistic pathways; they possessed a better ability to inhibit EGFR than erlotinib and a moderate activity on BRAF^{V600E} kinase. Upon correlating the activity to the structural modifications,

adding the oxime moiety boosted the activity dramatically. Moreover, importing pyrazine or substituted phenyl rings retained the overall activity.

Another important scaffold containing 3-benzyloxy-16-hydroxymethylene-estradiol was discovered by Zupkó and his research group [190]. Western blot assay showed that **64** significantly inhibited FAK phosphorylation in MDA-MB-231 cells in a concentration-dependent manner (Figure 21). Compound **64** possessed strong antiproliferative activity in five different breast cancer cells (MDA-MB-231, MCF7, T47D, MDA-MB-361 and MRC-5). Wound-healing assay revealed that estradiol **64** reduced the migration of 80% of MDA-MB-231 cells, indicating its substantial ability to prevent metastasis. This trial is an excellent example of incorporating the estradiol scaffold, which embodies an essential moiety used in hormonal-dependent cancers in FAK inhibition. In this respect, structural modifications could be performed for **64** to target both FAK and aromatase proteins that would fortify the anticancer activity.

Kang and coworkers [191] created two pharmacophore models to target FAK and cyclin-dependent kinase 4/6 (CDK4/6) active sites. CDKs play a crucial role in the process of mitosis and are overexpressed in various human tumors, and are considered an attractive target for the improvement of anticancer drugs [192]. Among seven million screened compounds, only six were selected from the pharmacophore model. Molecular docking and dynamics simulations performed on FAK and CDK4/6 revealed that **65** has an adequate molecular dynamics balance, stable structure and low docking energy (Figure 21). Docking of **65** within the ATP binding site of FAK revealed a hydrogen bond between the carbonyl group and Gly429, whereas the sulfur on thiazole made another hydrogen bond with Glu506. Regarding the CDK4/6 active site, the carbonyl group of **65** formed a hydrogen bond with Gly20, and the nitrogen atom on the piperazine ring made an ionic bond with Asp163 amino acid.

Conclusion

The present review article summarized many inhibitors created in the past three decades and provided an overview of the actions of FAK protein and FAK inhibitors. As an essential regulator of angiogenesis and cell migration, FAK is a proper target for cancer therapy. Numerous small-molecule FAK inhibitors were developed and assessed in preclinical trials and human clinical models. Some prepared FAK kinase inhibitors, such as **11**, **13** and **34**, could be ready for the preclinical trials due to their remarkable activity. Several attempts were made to enhance the anti-FAK activity, such as targeting both the ATP binding site and the allosteric binding site of FAK, FAK-FAT, and FAK-FERM active sites using different five- or six-membered nitrogen heterocyclic rings, dual and multiacting inhibitors, structure/fragment-based inhibitors, and PROTAC-mediated degradation. The strong antiproliferative nature of FAK inhibitors makes them attractive weapons against tumor cells if correctly targeted.

Future perspective: smarter FAK inhibitors

The diversity of the structural features of FAK inhibitors makes it a scalable route for the molecular hybridization approach. With the hope of slowing the emergence of drug resistance, several research groups investigated the dual or the multiacting activity of the anti-FAK derivatives. These trials targeted FAK with IGF-1R (TAE226, **1**), Pyk2 (PF-373228, **3**), ALK (**5**), EGFR^{T790M} (**12**), Akt (**30**) and CDK4/6 (**65**). All these dual-acting compounds targeted FAK and another kinase. Because the structure of the ATP-binding sites of most kinases share fundamental structural similarities, these dual kinase-targeting drugs are likely to have more off-target effects and side effects. For this reason, it might be best to combine nonkinase targets with FAK (e.g., histone deacetylase, aromatase, tubulin or topoisomerase); unlike kinase-inhibitors, the compounds that inhibit these nonkinase proteins display some of the highest degrees of specificity. It is likely that in the future this approach will move in the direction of PROTAC-oriented chimeric and multiacting drugs, with anti-FAK action supported by a scaffold(s) working by a different mechanism of action. The design of the chimeric compounds should then be used in PROTAC-mediated degradation recruited to target specific proteins.

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Executive summary

- FAK has been recognized as a promising target class for anticancer development.
- The FAK protein is comprised of FAT, central kinase and FERM domains.
- All FAK domains are targeted for FAK inhibition through binding to the ATP binding site or the phosphorylation sites.
- The ATP binding site of FAK contains two key residues: Cys502 of the kinase hinge and Asp564 of the DFG motif.
- The ATP binding site of FAK experiences conformational changes upon the inhibitor's binding.
- Several FAK inhibitors have been developed in the last three decades.
- Most of the developed inhibitors target the ATP binding site of the kinase domain (five- and six-membered inhibitors).
- Several trials targeted the FAK-FERM and FAK-FAT domains and the allosteric binding site.
- The developed FAK-proteolysis targeting chimeras (PROTAC) revealed promising activity and require further investigation.
- Targeting FAK and other nonkinase targets in one PROTAC compound could be a successful approach to pursue in next few years.

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