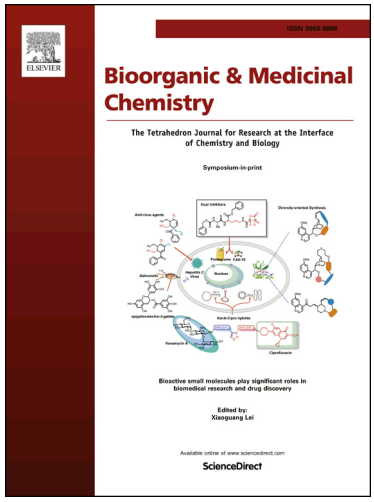

Design, synthesis and biological evaluation of sulfonamide-substituted diphenylpyrimidine derivatives (Sul-DPPYs) as potent focal adhesion kinase (FAK) inhibitors with antitumor activity

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Design, synthesis and biological evaluation of sulfonamide-substituted diphenylpyrimidine derivatives (Sul-DPPYs) as potent focal adhesion kinase (FAK) inhibitors with antitumor activity

Menghua Qu[a,1], Zhihao Liu[a,1], Dan Zhao[a], Changyuan Wang[a], Jianbin Zhang[a], Zeyao Tang[a], Kexin Liu[a], Xiaohong Shu[a,*], Hong Yuan[b,*], and Xiaodong Ma[a,*]

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Abstract

A class of sulfonamide-substituted diphenylpyrimidines (Sul-DPPYs) were synthesized to improve activity against the focal adhesion kinase (FAK). Most of these new Sul-DPPYs displayed moderate activity against the FAK enzyme with IC_{50} values of less than 100 nM; regardless, they could effectively inhibit several classes of refractory cancer cell lines with IC_{50} values of less than 10 μM, including the pancreatic cancer cell lines (AsPC-1, Panc-1 and BxPC-3), the NSCLC-resistant H1975 cell line, and the B lymphocyte cell line (Ramos cells). Results of flow cytometry indicated that inhibitor **7e** promoted apoptosis of pancreatic cancer cells in a dose-dependent manner. In addition, it almost completely induced the apoptosis at a concentration of 10 μM. Compound **7e** may be selected as a potent FAK inhibitor for the treatment of pancreatic cancer.

Key words: Cancer; FAK; inhibitor; pyrimidine; sulfonamide.

1. Introduction

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that localizes at sites of cell adhesion to the extracellular matrix (ECM) and mediates signaling events downstream of the integrin engagement of the ECM.[1,2] FAK played considerably important roles in regulating cancer cell survival, proliferation, and migration. It has also been identified as a key pathogenic mediator of various cancers. Inhibition of FAK activity has been demonstrated to affect a number of cellular processes essential for tumor growth and disease progression including angiogenesis and metastasis. Thus, FAK enzyme has emerged as a promising cancer therapeutic target.[3-5] Up to present time, a couple of FAK inhibitors have been described to undergo extensively preclinical or clinical trials for cancer therapy (Fig. 1), including TAE226 (**1**),[6] PF-573228 (**2**)[7-9], defactinib (**3**, phase II)[10], PF-562271 (**4**, phase I)[11,12], CEP-37440 (**5**, phase I)[13], and VS-4718 (**3**, phase I)[14],. Generally, most potent FAK inhibitors possess a pyrimidine core, along with a *C*-4 *N*-methylformamide-substituted aniline side chain, an important functionality to interact with amino acids Cys502, Asp546, and Leu553 in the FAK protein to tightly contact with FAK.[15]

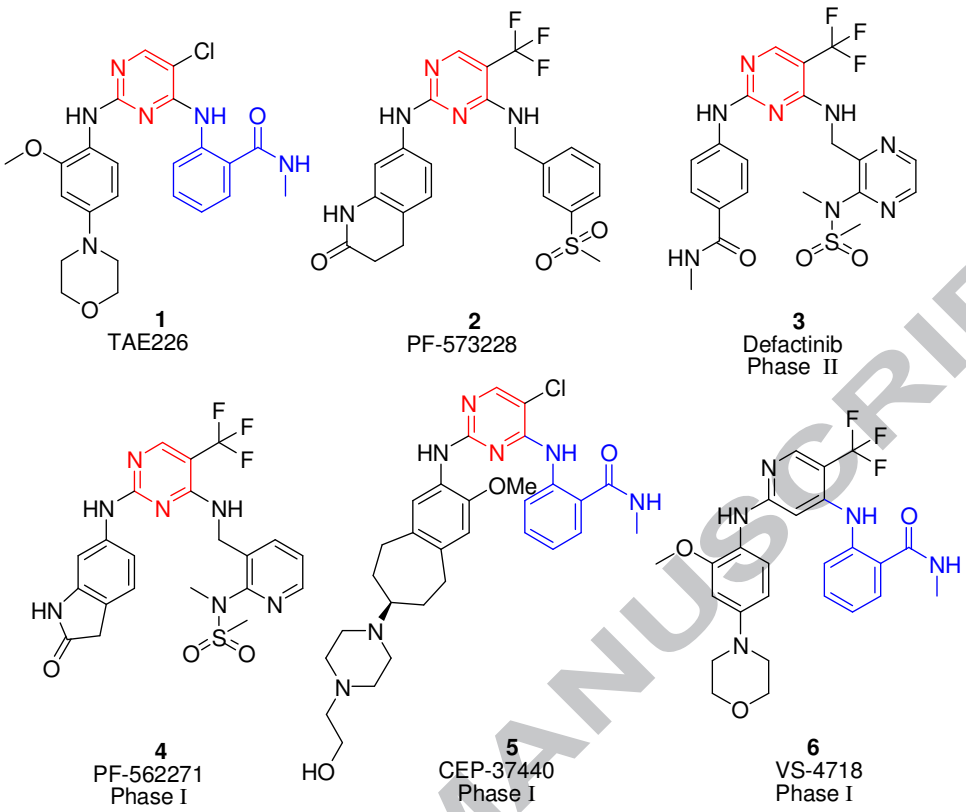


Figure 1. Chemical structures of the novel FAK inhibitors.

The sulfonamide functional group is present in many active compounds, exhibiting antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, and antithyroid properties, among other.[16-18] Our previous study revealed that introducing a functional sulfonamide into the *C*-2 aniline moiety of pyrimidine template could improve the activity against the Bruton's tyrosine kinase (BTK), which is an effective drug target for treatment of B-cell lymphoblastic leukemia.[19] According to the structure-based drug design strategy, a family of *C*-2 sulfonamide-substituted diphenylpyrimidine derivatives (Sul-DPPYs) featuring a TAE226 molecule template were synthesized to improve inhibitory potency toward FAK kinase activity and cancer cells in current study (Fig. 2). The synthesis and anticancer evaluation of these newly designed compounds were also reported in this manuscript.

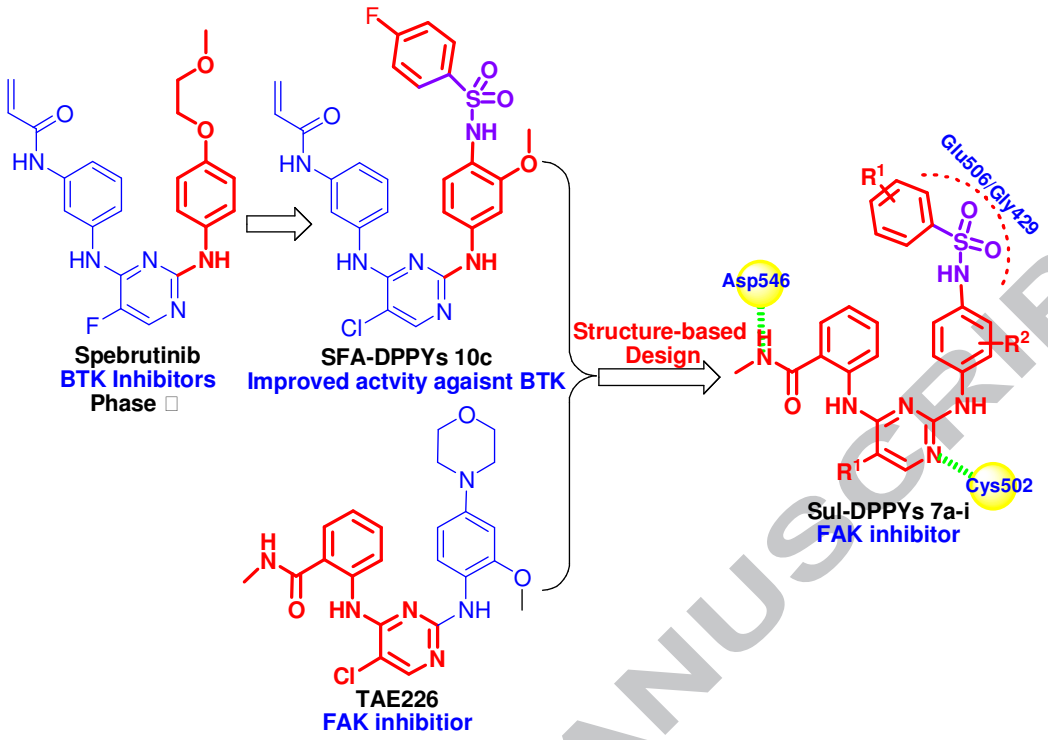
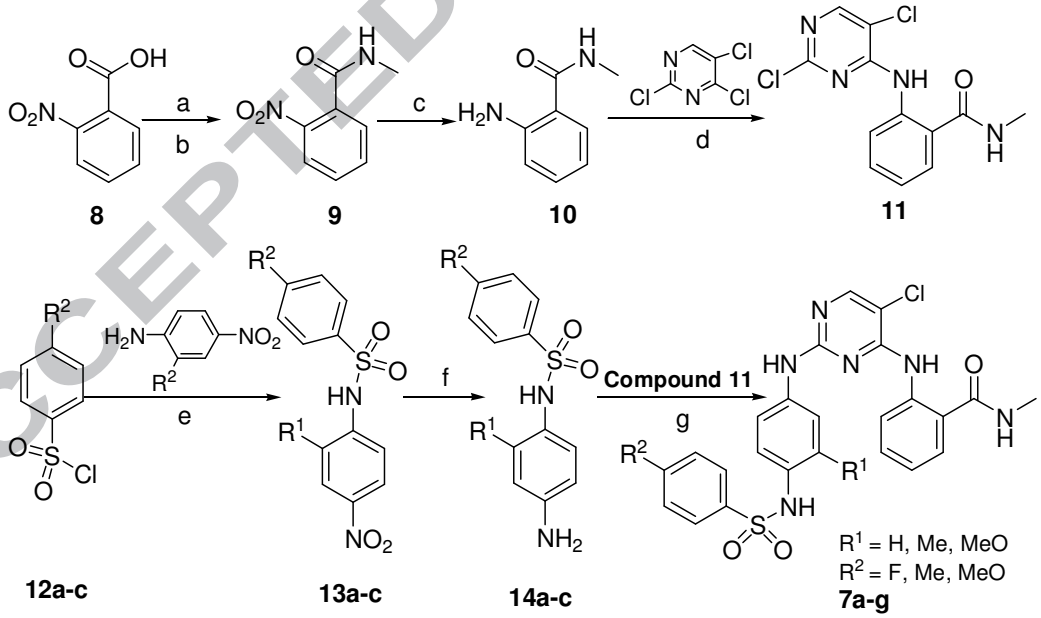


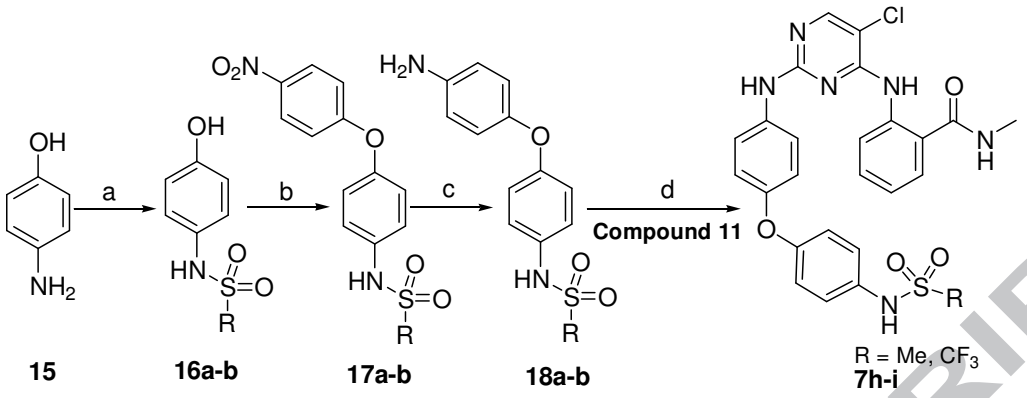
Figure 2. Designed strategy of the title molecules Sul-DPPYs.

2. Results and discussion

2.1. Chemistry



Scheme 1. Synthetic route of the title compounds **7a-g.** Reagents and conditions: (a) $SOCl_2$, 1 h, 60 °C; (b) $H_2NMe{\cdot}HCl$, $NaHCO_3$, CH_3CN , 4 h, 80 °C, 95%; (c) Fe-NH_4Cl, MeOH-H_2O, 2 h, 70 °C, 88%; (d) *N,N*-diisopropylethylamine (DIPEA), isopropanol, 6 h, 85%; (e) $NaHCO_3$, CH_3CN, r.t, 0.5 h, 90-95%; (f) Fe-NH_4Cl, MeOH-H_2O, 2 h, 70 °C, 84-88%; (g) Compound **11** trifluoroacetic acid, 2-BuOH, 100 °C, 12 h, 19-33%



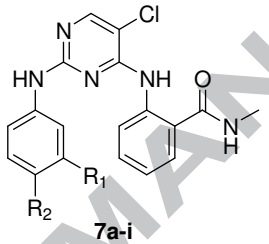
Scheme. 2. Synthetic route of the title compounds **7h-i.** Reagents and conditions: (a) CH_3SO_2Cl or CF_3SO_2Cl, $NaHCO_3$, CH_3CN , 4 h, 70 °C, 85-90%; (b) 4-fluoronitrobenzene, KI, K_2CO_3, DMF, 80 °C, 80-85%; (c) Fe-NH_4Cl, MeOH-H_2O, 2 h, 70 °C, 70-88%; (d) Compound **11**, *p*-toluenesulfonic acid, 2-BuOH, 100 °C, 12 h, 15-22%.

The designed molecules **7a-i** were synthesized according to the general procedures depicted in Scheme 1 and Scheme 2.[19-22] 2-Nitrobenzoic acid (**8**) was reacted with thionyl chloride, and then directly reacted with methylamine in the present of $NaHCO_3$ as base to produce 2-nitro-(*N*-methyl)benzamide (**9**). Compound **9** was then reduced to 2-amino-substituded *N*-methylbenzamide (**10**) by using the Fe-NH_4Cl reduction reagent. Subsequently, compound **10** was region selectively coupled with the *C*-4 chlorine atom in the 2,4,5-trichloropyrimidine reagent to form the 2-chloropyrimidine scaffold **11**. Aniline intermediates **14a-c** were prepared from benzenesulfonyl chloride derivatives by subsequent reaction with 4-nitroanilene and the Fe-NH_4Cl reagent. By the nucleophilic substitution reaction of 2-chlorine-substituted pyrimidine **11** with anilines **14a-c** under the *p*-toluenesulfonic acid condition, the desired molecules **7a-g** were synthesized. Applying a similar synthetic strategy, another family of the title compounds **7h-i** were also prepared shown in Scheme 2. Generally, 4-aminephenol was converted to *N*-phenyl methanesulfonamide intermediates **16a-b** under the action of methanesulfonyl chloride or trifluoromethane sulfonic anhydride. Then **16a-b** were reacted with 4-fluoronitrobenzene to form the intermediates **17a-b**, which were conveniently converted to amine derivatives **18a-b** by using the Fe-NH_4Cl reduction condition. Also under the action of *p*-toluenesulfonic acid reagent, **18a-b** was reacted with 2-chlorine pyrimidine **11** to generate the title molecules **7h-i**.

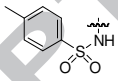
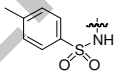
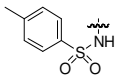
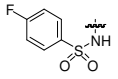
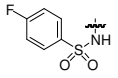
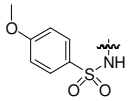
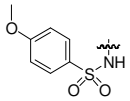
2.2. Biological activity

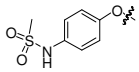
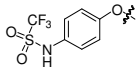
The capacity of these newly obtained compounds for inhibiting FAK enzyme activity was explored by ADP-Glo™ Kinase Assay,[23,24] The antiproliferative activity against cancer cells was determined by CCK-8 testing.[25] The effects on cell apoptosis were analyzed by flow cytometry. For these biological evaluation, the human pancreatic carcinoma cell line (AsPC-1, Panc-1, BxPC-3), typical B-cell lymphoblastic leukemia cell line (Ramos), and gefitinib-resistant lung cancer cell line (H1975), which harbored overexpressed FAK protein, were employed. Human normal liver cell line (LO-2) was also used as a negative selection for comparison, Gemcitabine, a chemotherapy medication used to treat several types of cancer, was selected as a reference compound.

Table 1. Biological activity of the newly synthesized compounds **7a-i**.[a]



7a-i

Compd.	R_1	R_2	FAK Enzymatic activity (IC_{50}, nM)[b]	Antiproliferative activity (IC_{50}, μM)					
				AsPC-1	Panc-1	BxPC-3	Ramos	H1975	L0-2
7a	Me		84.6	2.32	2.99	23.1	20.3	9.56	27.5
7b	H		82.5	1.55	2.76	19.4	6.17	7.07	28.7
7c	MeO		109.2	10.1	> 40	22.5	12.1	8.50	> 40
7d	MeO		84.7	6.69	5.70	31.6	24.6	12.91	19.7
7e	H		86.7	3.92	0.53	6.92	2.18	3.71	11.5
7f	H		110.0	3.49	1.80	24.1	9.10	4.28	8.37
7g	Me		113.8	1.78	0.03	10.6	4.61	4.89	2.88

7h	H		108.8	2.75	3.61	14.2	19.8	6.29	16.5
7i	H		235.9	2.39	1.75	5.86	14.4	4.10	3.37
GCT[c]				26.8	30.4	20.2	>10	>10	>20
TAE 226			6.79	6.73	>20	1.03	--[d]	--	5.37

[a] Data represent the mean of at least three separate experiments. [b] Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations in micromolar needed to inhibit cell growth by 50%, as calculated using GraphPad Prism version 5.0. [c] GCT: Gemcitabine. [d] Untested.

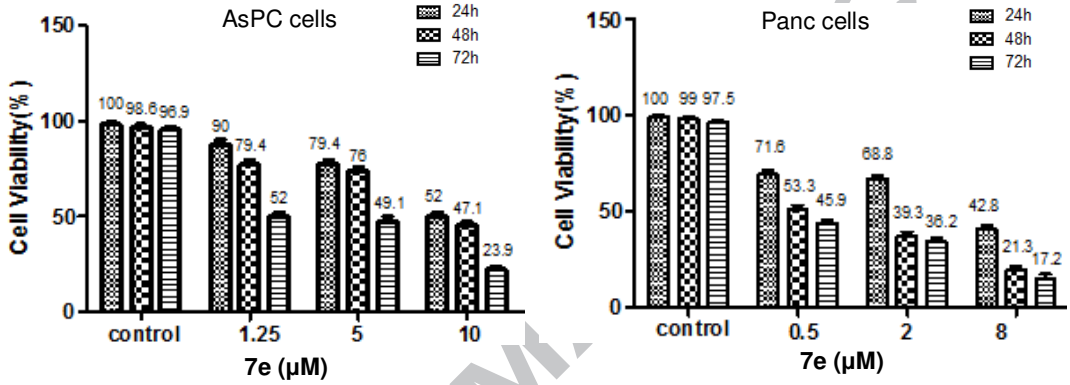


Figure 3. Time and concentration dependant effects of compound **7e** on the antiproliferative activity of Panc-1 and AsPC-1 cell lines. Results are representative of three separate experiments, dates are expressed as the mean ± standard deviation, $p < 0.05$.

Sul-DPPYs were initially assessed for their ability to inhibit FAK enzymatic activity. The *in vitro* IC_{50} values are summarized in Table 1. The kinase-based test results indicate that all these compounds exhibit moderate inhibitory activity against the FAK enzyme within nanomolar concentrations. Among them, 4 compounds exhibited strong anti-FAK activity at concentrations lower than 100 nM: **7a**, **7b, 7d** and **7e**. Apparently, the trifluoromethanesulfonyl group is unfavorable for improving the inhibitory activity against FAK. The exemplary compound **7i,** with an IC_{50} value of 235.9 nM, was considered the least active inhibitor of these compounds. In cell-based assays, a large portion of Sul-DPPYs could effectively inhibit the proliferation of these cancer cell lines at concentrations ranging from 0.03 to 40 μM. In particular, compounds **7e**, **7g,** and **7i** exhibited excellent capabilities to interfere with the proliferation of all cancer cell lines within concentrations of 15 μM. Compounds **7e** and **7g** possessed sensitivity that allows them to inhibit Panc-1 cell activity, with IC_{50} values of 0.53 and 0.03 μM, respectively. By contrast, the reference drug gemcitabine

has an IC_{50} value of more than 20 μM against the AsPC-1, Panc-1, and BxPC-3 cell lines. This finding suggests that Sul-DPPYs are more active than gemcitabine against pancreatic cancer. All Sul-DPPYs also showed low cytotoxic activity against the normal liver cell line LO-2, indicating that Su-DPPYs exert less effect on LO-2 cells than the cancer cells. Typically, compound **7e**, still possesses low cytotoxicity against normal LO-2 cells (IC_{50}=11.5 μM). Although compound **7e** is less active against FAK enzyme, it possesses strong potency to inhibit pancreatic carcinoma cell lines, and low toxicity against LO-2 cells. Therefore, inhibitor **7e** may be selected as a new valuable lead compound to discover more effective drugs for treatment of pancreatic cancer.

Compound **7e**, the active inhibitor, was selected to evaluate the inhibition of cell viability in Panc-1 and AsPC-1 cell lines. As presented in Figure 3, compound **7e** apparently inhibited cell viability for Panc-1 at concentrations ranging from 0.5 μM to 8 μM and for Aspc-1 from 1.25 to 10 μM. In particular, cell viability was inhibited up to 50% at 72 h for all the concentrations. With increases in time and concentration, the viability rate was suppressed by 82.8% for Panc-1 cells and 76.1% for AsPC-1 cells.

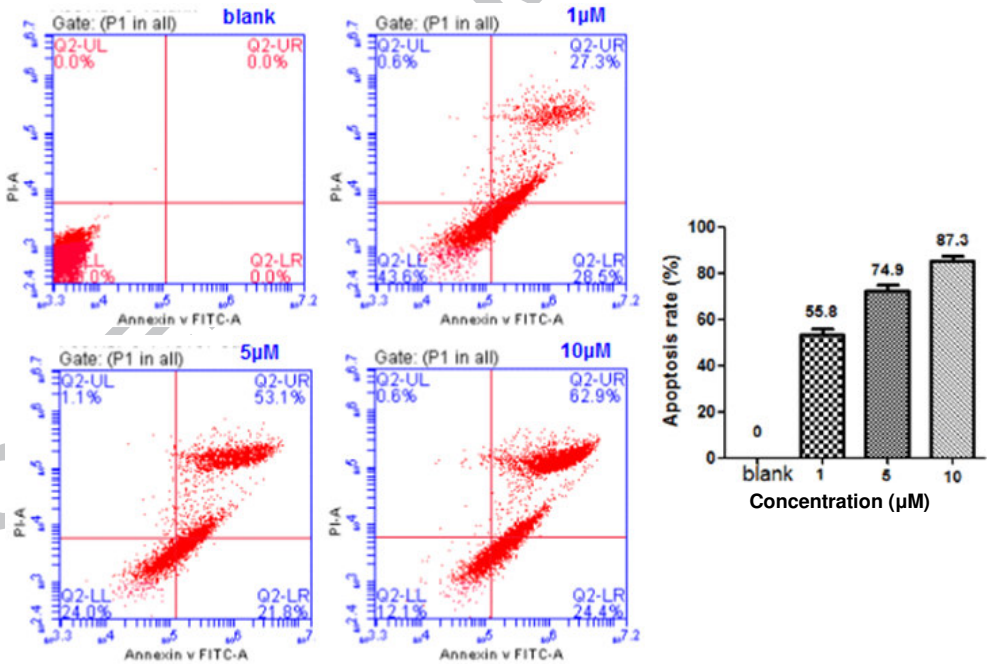


Figure 4. Compound **7e** induced Aspc-1 cell apoptosis *in vitro*. The cells were incubated with the indicated concentrations of **7e** for 72 h, and the cells were stained with annexin V/FTIC, followed by flow cytometry analysis. One representative experiment is shown.

2.3. Flow cytometry

To further investigate the antiproliferative mechanism of these inhibitors on pancreatic cancer cells, the effects of inhibitor **7e** on apoptosis in the AsPC-1 cell line was evaluated by flow cytometry (Fig. 4). Compound **7e** strongly induced the apoptosis of AsPC-1 cells, with apoptotic rates of 55.8%, 74.9%, and 87.3% at concentrations of 1, 5, and 10 μmol/L, respectively. These results indicated that the percentages of cell apoptosis correspond to the concentrations of the compounds, which confirmed that compound **7e** could induce apoptosis in a dose-dependent manner.

2.4. Molecular simulations

To explore the putative interaction mechanism of Sul-DPPYs with FAK enzyme, the active inhibitor **7e** and the less active inhibitor **7i** were docked into the binding pocket of the FAK enzyme (PDB code: 2JKK) by using Autodock4.2,[26] respectively. The docking models are shown in Figure 5. AutoDock 4.2 with its default parameters was used.[27,28] The novel FAK inhibitor TAE226 was also analyzed using the same procedure for comparison (Fig. 5A).

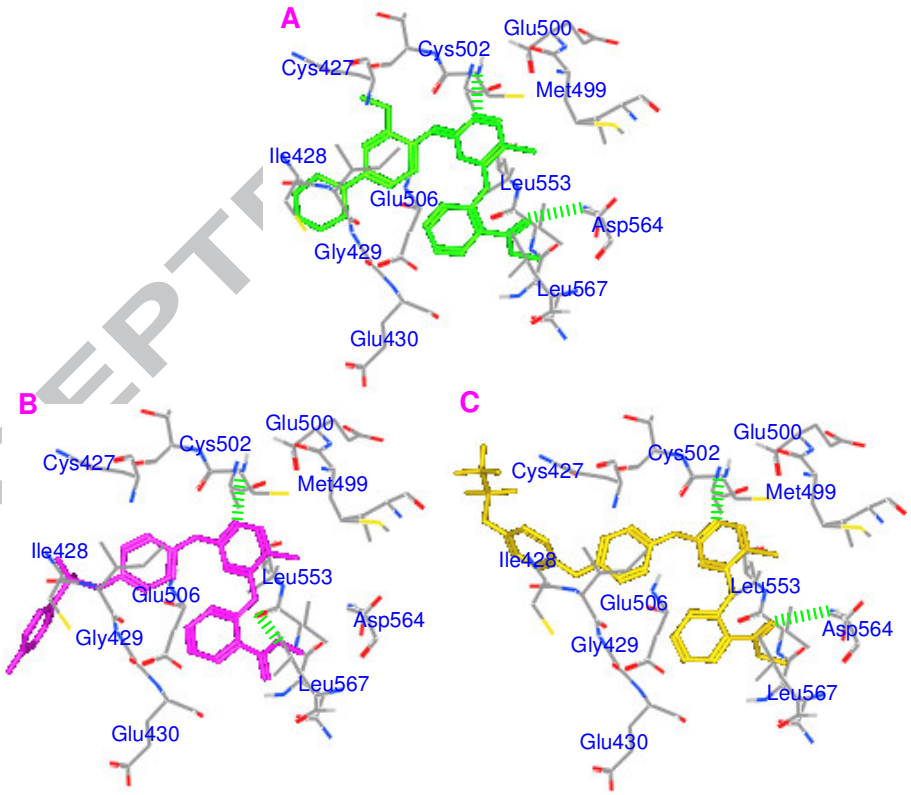


Figure 5. Predicted binding pose of the typical inhibitors in FAK enzyme (PDB code: 2JKK), A: TAE226, B: inhibitor **7e**; C:inhibitor **7i**.

The binding model clearly indicated that inhibitors **7e** (Fig. 5B) and **7i** (Fig. 5C) interact with the FAK enzyme similarly to TAE226 (Fig. 5A). All of them formed several important contacts with the FAK protein below: (1) hydrogen-bond forces between the *N*-1 atom of the pyrimidine core with the amino acid Cys502 for TAE226 and inhibitor **7i**, whereas the hydrogen bond comes from the NH group in amide functionality with Leu553 for **7e**; (2) van der Waal contacts produced by the *C*-5 chlorine atom with the amino acid Met499; (3) dipole-induced dipole interactions formed by the hydrophilic section of the binding pocket surrounded with amino acids Ile428 and Glu429 with the morpholine substituent in TAE226, and the sulfonamide group in inhibitor **7e**. However, for inhibitor **7i**, these polar forces disappeared because the sulfonamide substituent moved far away from the hydrophilic binding section. Instead, the hydrophobic phenyl ring in molecules **7e** and **7i** occupied the hydrophilic section, which possesses unfavorable characteristics that hinder interaction with the FAK enzyme. To summarize, this binding model exactly explained their anti-FAK activity.

3. Conclusion

A new class of diphenylpyrimidine derivatives with a sulfonamide functionality were synthesized and biologically evaluated as potent FAK inhibitors. The typical inhibitor **7e** effectively inhibited FAK activity with an IC_{50} value of 86.7 nM. Notably, inhibitor **7e** significantly suppressed the proliferation of the pancreatic cancer cell lines AsPC-1and Panc-1 at concentrations of 3.92 and 0.53 μM, respectively. Flow cytometry results indicated that **7e** promoted the apoptotic rate of AsPC cells, which is 87.3% at a concentration of 10 μM. In addition, molecular simulation results suggested that **7e** binds tightly with the FAK enzyme. These results provided a theoretical basis for the further structural optimization of sulfonamide-substituted derivatives as FAK inhibitors and demonstrated that compound **7e** could potentially inhibit FAK for the treatment of pancreatic cancer.

4. Experimental section

4.1. General methods and chemistry

Reagents were obtained from commercial suppliers and used without further purification. Solvents were purified and stored according to standard procedures. All reactions were monitored by TLC, using silica gel plates with fluorescence F254

(TLC Silica gel 60 F254, Merck) and UV light visualization. High resolution ESI-MS were performed on an AB Sciex TripleTOF® 4600 LC/MS/MS system. ^{1}H NMR and ^{13}C NMR spectra on a Brucker AV 400 MHz spectrometer were recorded in [*d*]DMSO. Coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to internal control (TMS). ^{1}H NMR spectra are reported in the following order: multiplicity, approximate coupling constant (*J* value) in hertz, and number of protons; signals are characterized as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet) and br (broad signal). Flash chromatography separations were obtained on silica gel (300–400 mesh) using dichloromethane/methanol as eluents.

4.2.1. Preparation of the 2-chlorine pyrimidine 11.[19,20]

2-Amino-*N*-methylbenzamide **10** was prepared according to the general procedure in literature 19. The prepared 2-amino-*N*-methylbenzamide **10** (10.00 g, 66.67 mmol) was added in one portion to 2,4,5-trichloropyrimidine (12.23 g, 66.67 mmol) and *N,N*-diisopropylethylamine (DIPEA) (12.93 g, 100 mmol) in isopropanol (100 mL). The resulting mixture was stirred at 80 °C for 6 hours. The mixture was evaporated to dryness. The organic phase was evaporated to dryness and the residue was recrystallised from MeCN/water 20:1 to yield 2-(2,5-dichloropyrimidin-4-ylamino)-*N*-methylbenzamide 16.87 g, 85 %.

4.2.2. Preparation of the title molecules7a-h.[21,22]

Anilines **14a-c** and **18a-b** were synthesized using the reported method in literatures 21. The obtained compounds **14a-c** or **18a-b** (1 mmol), **11** (1 mmol), TFA (1.5 mmol), and 2-BuOH (20 mL) was mixed in a round-bottom flask. The slurry was heated to 100 °C for 5 h. The reaction mixture was allowed to cool to room temperature and was neutralized with a saturated aqueous sodium bicarbonate solution. The aqueous mixture was then extracted with CH_2Cl_2 (20 mL) three times. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to obtain a crude product. The residue was purified by column chromatography on silica gel (elution with 5% methanol /dichloromethane) to give the title product.

4.2.2.1. *N*-(3-((5-Nitro-2-(4-(3-(4-methylbenzenesulfonicamide)methyl)phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10a**). Yield: 25.3%; off-brown solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.90 (s, 3H), 2.37 (s, 3H), 2.80 (d, J = 4.0 Hz, 3H), 6.80 (d, J = 12.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.31-7.36 (m, 4H), 7.42 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 1H), 8.20 (s, 1H), 8.65 (d, J = 8.0 Hz, 1H), 8.78 (d, J = 4.0 Hz, 1H), 9.35 (s, 1H), 9.44 (s, 1H), 11.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.42, 21.48, 26.79, 105.6, 117.9, 121.2, 121.7 (2C), 122.3, 127.1 (2C), 127.8, 128.5, 128.8, 130.0 (2C), 131.8, 135.4, 138.3, 139.1, 139.7, 143.3, 151.1, 153.4, 158.0, 169.3; HRMS (ESI) for $C_{26}H_{25}ClN_6O_3S$; $[M+H]^+$ calcd: 537.1470; found: 537.1443.

4.2.2.2. *N*-(3-((5-Nitro-2-(4- (4-methylbenzenesulfonicamide)phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10b**). Yield: 27.5%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.35 (s, 3H), 2.81 (d, J = 4.0 Hz, 3H), 6.99 (d, J = 12.0 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 7.31-7.37 (m, 3H), 7.50 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 8.20 (s, 1H), 8,67 (d, J = 4.0 Hz, 1H), 8.81 (d, J = 4.0 Hz, 1H), 9.43 (s, 1H), 10.0 (s, 1H), 11.64 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.44, 26.79, 105.5, 121.0, 121.1, 121.8, 122.1 (2C), 122.4, 126.0, 127.2 (2C), 128.5, 130.0 (2C), 131.7, 132.0. 137.2, 137.4, 139.8, 143.5, 155.1, 155.4, 158.1, 169.4; HRMS (ESI) for $C_{25}H_{23}ClN_6O_3S$; $[M+H]^+$ calcd: 523.1314; fou methoxyl nd: 523.1281.

4.2.2.3. *N*-(3-((5-Nitro-2-(4-(3-(4-methylbenzenesulfonicamide)methoxyl)phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10c**). Yield: 32.8%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.37 (s, 3H), 2.82 (d, J = 4.0 Hz, 3H), 3.33 (s, 3H),7.07 (d, J = 8.0 Hz, 1H), 7.15 (t, J = 8.0 Hz, 1H), 7.23 (t, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 3H), 7.59 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 1H), 8.24 (s, 1H), 8.71-8.83 (m, 2H), 9.26 (s,1H), 9.48 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.44, 26.79, 55.56, 104.0, 105.8, 111.8, 119.3, 121.1, 121.6, 122.4, 126.9, 127.3 (2C), 128.5, 129.6 (2C), 131.9, 138.4, 139.8, 139.9, 143.0, 153.6, 155.0, 155.4, 158.0, 169.4; HRMS (ESI) for $C_{26}H_{25}ClN_6O_4S$; $[M+H]^+$ calcd: 553.1419; found: 553.1382.

4.2.2.4 *N*-(3-((5-Chloro-2-(4-(3-(4-fluorinebenzenesulfonicamide)methoxyl)phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10d**). Yield: 25.9%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.82 (d, J = 4.0 Hz, 3H), 3.30 (s, 3H), 7.07 (d, J = 8.0 Hz, 1H), 7.13 (t,

J = 8.0 Hz, 1H), 7.21-7.27 (m, 2H), 7.39 (t, *J* = 8.0 Hz, 3H), 7.71-7.78 (m, 3H), 8.23 (s, 1H), 8.70 (d, *J* = 8.0 Hz, 1H), 8.80 (d, *J* = 4.0 Hz, 1H) 9.42 (s, 1H), 9.48 (s, 1H), 11.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.79, 55.44, 103.8, 105.9, 111.8, 116.1, 116.4, 118.7, 121.1, 121.7, 122.4, 127.9, 128.5, 130.2 (d, *J* = 40 Hz, 1C), 131.9, 137.6 (d, *J* = 12.0 Hz, 1C), 139.8, 140.3, 153.9, 155.0, 155.4, 157.9, 163.3, 165.8, 169.4; HRMS (ESI) for $C_{25}H_{22}ClFN_6O_4S$; $[M+H]^+$ calcd: 557.1169; found: 557.1147.

4.2.2.5 *N*-(3-((5-Chloro-2-(3-(4-fluorinebenzenesulfonicamide) phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10e**). Yield:31.8%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.82 (d, *J* = 4.0 Hz, 3H), 6.97 (d, *J* = 8.0 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.75-7.80 (m, 3H), 8.19 (s, 1H), 8.66 (d, *J* = 4.0 Hz, 1H), 8.78 (d, *J* = 4.0 Hz, 1H), 9.44 (s, 1H), 10.06 (s, 1H), 11.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.79, 105.5, 116.7, 117.0, 120.9, 121.2, 121.8, 122.4, 122.5 (2C), 135.4, 128.5, 130.22 (d, *J* = 40 Hz, 1C), 131.5, 131.7, 136.3 (d, *J* = 12 Hz, 1C), 137.8, 139.7, 155.1, 155.4, 158.1, 163.5, 166.0, 169.3; HRMS (ESI) for $C_{24}H_{20}ClFN_6O_3S$; $[M+H]^+$ calcd: 527.1063; found: 527.1044.

4.2.2.6 N-(3-((5-Nitro-2-(4-(3-(4-methoxylbenzenesulfonicamide)methyl)phenylamino)-4-pyrimidinyl)amino)phenyl)-N-methylbenzamide (7f) Yield: 29.9%; off-brown solid; 1HNMR (400 MHz, DMSO-d6): δ 1.93 (s, 3H), 2.51 (s, 3H), 2.82 (d, J = 4.0 Hz, 3H), 6.80 (d, J = 8.0 Hz, 1H), 7.10-7.14 (m, 3H), 7.30-7.36 (m, 2H), 7.43(s, 1H), 7.58 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H), 8.66 (d, J = 8.0 Hz, 1H), 8.81 (d, J = 4.0 Hz, 1H), 9.28 (s,1H), 9.45 (s, 1H), 11.62 (s, 1H); 13C NMR (400 MHz, DMSO-d6): δ 18.44, 26.79, 56.11, 105.6, 114.7 (2C), 117.9, 121.2, 121.7, 122.3, 127.8, 128.5, 129.0, 129.3 (2C), 131.9, 132.8, 135.4, 139.0, 139.7, 155.2, 155.4, 158.0, 162.7, 169.4; HRMS (ESI) for $C_{26}H_{25}ClN_6O_4S$; [M+H]+ calcd: 553.1419; found: 553.1411.

4.2.2.7 *N*-(3-((5-Chloro-2-(4-(4-methoxylbenzenesulfonicamide)phenylamino)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10g**). Yield: 19.7%; off-yellow solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.52 (s, 3H), 2.82 (d, *J* = 4.0 Hz, 3H), 6.99 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.16 (t, *J* = 4.0 Hz, 1H), 7.30-7.34 (m, 1H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 8.20 (s, 1H), 8.68 (d, *J* = 8.0 Hz, 1H), 8.83 (s,1H), 9.43 (s, 1H), 9.97 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.78, 56.06, 105.4, 114.7 (2C), 121.0, 121.11, 121.8, 122.0 (2C), 122.3, 128.5, 129.4 (2C), 131.7, 131.8 (2C), 132.2, 137.28, 139.75,

155.1, 155.4, 158.1, 162.7, 169.4; HRMS (ESI) for $C_{25}H_{23}ClN_6O_4S$; $[M+H]^+$ calcd: 539.1263; found: 539.1249.

4.2.2.8 *N*-(3-((5-Chloro-2-(4-(4-methylsalfonamido)phenoxy)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10h**). Yield: 25.3%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.51 (s. 3H), 2.81 (d, J = 4.0 Hz, 3H), 6.97 (dd, J = 4.0 Hz, 12.0 Hz, 4H), 7.13 (t, J = 8.0 Hz, 1H), 7.22 (d, J = 12.0 Hz, 2H), 7.46 (t, J = 8 Hz, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H), 8.73-8.82 (m, 2H), 9.49 (s, 1H), 9.62 (s, 1H), 11.63 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.79 (2C), 105.5, 119.1 (2C), 119.5 (2C), 121.2, 121.9, 122.0, 122.4, 133.2 (2C), 128.5, 130.1, 131.9, 133.6, 136.6 139.8, 151.5, 154.8, 155.1, 155.5, 158.2, 169.4; HRMS (ESI) for $C_{25}H_{23}ClN_6O_4S$; $[M+H]^+$ calcd: 539.1263; found: 539.1229.

4.2.2.9 *N*-(3-((5-Chloro-2-(4-(4-trifluoromethylsalfonamido)phenoxy)-4-pyrimidinyl)amino)phenyl)-*N*-methylbenzamide (**10i**). Yield: 21.8%; off-brown solid; ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.81 (d, J = 4.0 Hz, 3H), 6.90 (d, J = 4.0 Hz, 2H), 6.96 (d, J = 4.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 4H), 7.45 (t, J = 8.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 8.21 (s, 1H), 8.73 (d, J = 4.0 Hz, 1H), 8.79 (d, J = 8.0 Hz, 1H) 9.48 (s, 1H), 11.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.78, 105.4, 118.7 (2C), 118.8, 119.4 (2C), 119.7, 121.2, 121.9, 122.0, 122.4, 122.9, 125.6 (2C), 128.5, 131.9, 136.5, 139.7, 151.6, 154.4, 155.1, 155.5, 158.3, 169.4; HRMS (ESI) for $C_{26}H_{20}ClF_3N_6O_4S$; $[M+H]^+$ calcd: 593.0980; found: 593.0960.

4.3. In vitro kinase enzymatic assay

The FAK kinase enzyme system (Catalog. V9301) , purchased from Promega Corporation (USA), was used to test the activity according to the instructions of the manufacturer. Concentrations consisting of suitable levels from 10 to 500 nM were used for all of the tested compunds. The more detailed and complete protocols can be founded in the ADP-Glo™ kinase Assay Technical Manual available at: http://cn.promega.com/resources/protocols/product-information-sheets/n/btk-kinase-enzyme-system-protocol/. The test was performed in a 384-well plate, and includes the major steps below: (1) perform a 5 μL kinase reaction using 1× kinase buffer (e.g., 1× reaction buffer A), (2) incubate at room temperature for 60 minutes, (3) add 5 μL

of ADP-Glo™ Reagent to stop the kinase reaction and deplete the unconsumed ATP, leaving only ADP and a very low background of ATP, (4) incubate at room temperature for 40 minutes, (5) add 10 μL of Kinase Detection, (6) reagent to convert ADP to ATP and introduce luciferase and luciferin to detect ATP, (7) incubate at room temperature for 30 minutes, (8) plate was measured on TriStar® LB942 Multimode Microplate Reader (BERTHOLD) to detect the luminescence (Integration time 0.5-1 second). Curve fitting and data presentations were performed using GraphPad Prism version 5.0.

4.4 . Cellular activity assay

4.4.1. Cell culture and reagents

Ramos, AsPC-1, Panc-1, BxPC-3, H1975, and L0-2 cells were purchased from Fuheng Biology Company (Shanghai, China). The Cell Counting Kit-8 (CCK-8) reagent was purchased from Biotool Company (Switzerland). The Annexin V-FITC Apoptosis Detection Kit and Cell Cycle Assay were purchased from Beyotime Company (China). All cell lines were grown in RPMI-1640 (Gibco®, USA) supplemented with 10% FBS (Gibco®, USA), 1% penicillin-streptomycin (Beyotime Company, China). The cells were maintained and propagated as monolayer cultures at 37 °C in humidified 5% CO_2 incubator.

4.4.2. Cellular activity assay

CCK-8 method assay method was used to test the cell viability. Cells were seeded in 96-well plates at a density of 3,000 to 5,000 cells/well and were maintained at 37 °C in a 5% CO_2 incubator in RPMI1640 containing 10% FBS for one day. Cells were then exposed to the compouds for 72 h, and the number of cells used per experiment for each cell lines was adjusted to obtain an absorbance of 0.5 to 1.2 at 450 nm with a microplate reader (Thermo, USA). Compounds were tested at appropriate concentrations (1.25 to 40 μmol/L), with each concentration duplicated five times. Gemcitabine was employed as the positive control. The IC_{50} values were calculated using GraphPad Prim version 5.0.

4.5. Cell Apoptosis Assay

AsPC-1 cells (2 to 5 $\times 10^5$ cells/well) incubated in 6-well plates were treated with solvent control (DMSO), or compound **7e** (1, 5 or 10 μM) in medium containing 5% FBS for 72 h. Then, collected and fixed with 70% ethanol at 4 °C overnight. After being fixed with 75% ethanol at 4 °C for 24 h, the cells were stained with Annexin V-FITC (5 μL)/propidium iodide (5 μL), and analyzed by flow cytometry assay (Becton-Dickinson, USA).

4.6. Molecular docking study

The AutoDock 4.2 software with its default parameters was used to perform the docking exploration. Detailed tutorials that guide users through basic AutoDock usage, docking with flexible rings, and virtual screening with AutoDock may be found at: http://autodock.scripps.edu/faqs-help/tutorial. Generally, the crystal structure (PDB: 2JKK) of the kinase domain of FAK bound to inhibitor **4** was used in the docking studies. The enzyme preparation and the hydrogen atoms adding was performed in the prepared process. The whole EGFR enzyme was defined as a receptor and the site sphere was selected on the basis of the binding location of TAE226. By moving TAE226 and the irrelevant water, molecule **7e.** The binding interaction energy was calculated to include Van der Waals, electrostatic, and torsional energy terms defined in the tripos force field. The structure optimization was performed using a genetic algorithm, and only the best-scoring ligand protein complexes were kept for analyses.

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Graphical Abstract

Design, synthesis and biological evaluation of sulfonamide-substituted diphenylpyrimidine derivatives (Sul-DPPYs) as potent focal adhesion kinase (FAK) inhibitors with antitumor activity

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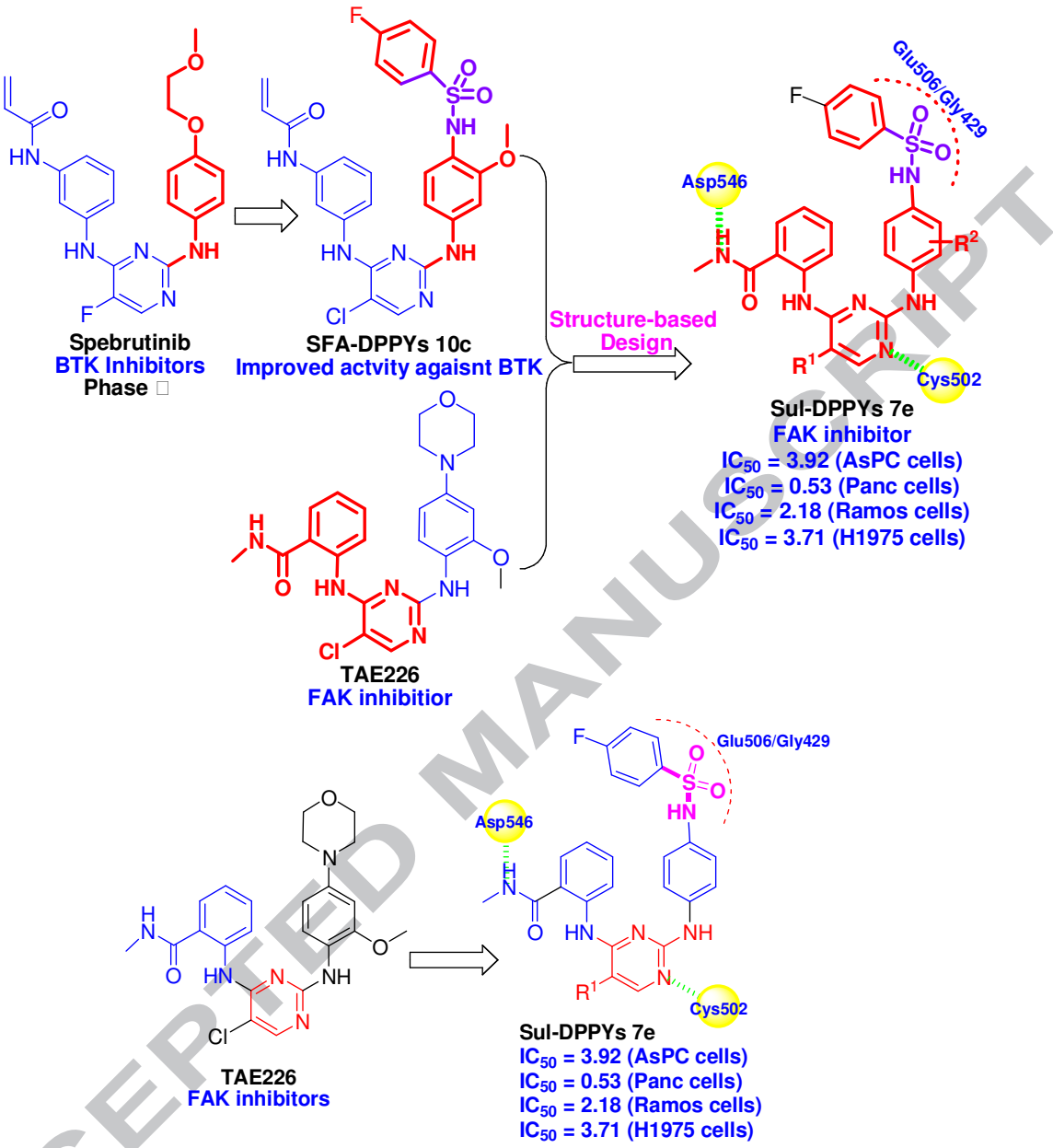

Highlights

- Sulfonamide-substituted diphenylpyrimidines (Sul-DPPYs) were synthesized as FAK inhibitors
- Sul-DPPYs could effectively inhibit pancreatic cancer cell lines and NSCLC-resistant H1975 cell line (IC_{50} < 10μM).
- Compound **7e** promoted apoptosis of pancreatic cancer cells in a dose-dependent manner.
- Compound **7e** may be selected asis a potential FAK inhibitor for treatment of pancreatic cancer.