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Graphical Abstract

Design, synthesis and biological evaluation of pyrazolylaminoquinazoline derivatives as highly potent pan-fibroblast growth factor receptor inhibitors

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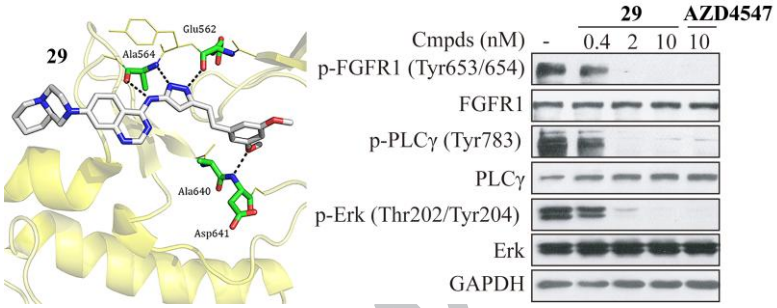
Jun Fan[a,#], Yang Dai[b,#], Jingwei Shao[a], Xia Peng[b], Chen Wang[c], Sufen Cao[d], Bin Zhao[a], Jing Ai[b,*], Meiyu Geng[b,*], Wenhu Duan[a,*]



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[b]*Division of Antitumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), 555 Zu Chong Zhi Road, Shanghai 201203, China*
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ABSTRACT

Fibroblast growth factor receptors (FGFRs) are important oncology targets due to the dysregulation of this signaling pathway in a wide variety of human cancers. We identified a series of pyrazolylaminoquinazoline derivatives as potent FGFR inhibitors with low nanomolar potency. The representative compound **29** strongly inhibited FGFR1–3 kinase activity and suppressed FGFR signaling transduction in FGFR-addicted cancer cells; FGFRs-driven cell proliferation was also strongly inhibited regardless of mechanistic complexity implicated in FGFR activation, which further confirmed that **29** was a potent pan-FGFR inhibitor. The flexibility of our structure offered the potential to preserve good affinity for mutant FGFR, which is important for developing TKIs with long-term efficacy.

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Fibroblast growth factor receptors (FGFRs) are transmembrane receptor tyrosine kinases (RTKs).[1] Binding of FGFRs to their cognate ligands fibroblast growth factors (FGFs) in concert with heparin sulfate proteoglycans (HPSG) activates FGF/FGFR signaling pathway, leading ultimately to the upregulation of several downstream signaling pathways including MEK1/2-ERK1/2, PLCγ, etc.[1] FGF/FGFR signaling plays a ubiquitous role in cell proliferation, differentiation, migration and survival.[1-6] In cancer, constitutive FGFR signaling is activated by point mutations, gene amplification, or chromosomal translocations/rearrangements.[6] Dysregulation of FGFR signaling has been documented in clinical samples of bladder, breast, and gastric cancers and so on.[1, 6-11] To date many methods have been adopted to generate agents to disrupt FGF-ligand/receptor activity, including monoclonal antibodies, FGF-ligand traps, and small-molecule tyrosine kinase inhibitors (TKIs).[12] The most clinically advanced of these agents are mainly mixed kinase inhibitors such as lucitanib,[13] dovitinib,[14-15] brivanib,[16-17] and ponatinib;[18] they exert anti-FGFR activity but the predominant activity is inhibition of vascular endothelial growth factor receptor 2 (VEGFR2), which compromises the FGFR selectivity and may also bring about toxicity issues. Selective FGFR TKIs have begun to emerge in recent years, and several inhibitors have progressed robustly into clinical trials, such as NVP-BGJ398,[19] AZD4547,[20] JNJ-42756493, TAS-120, CH-5183284,[21] and LY-2874455.[22]

AZD4547 inhibits recombinant FGFR kinase activity *in vitro* at the low nanomolar level, along with favorable selectivity over VEGFR2,[20] and it is currently in phase II clinical trials. Compound **1** (Figure 1) was another potent and selective FGFR inhibitor developed by AstraZeneca.[23] The crystal structure of **1** with FGFR1 revealed that **1** formed a U-shaped configuration to bind to the ATP binding pocket of FGFR1 (PDB ID: 4NK9)[24]: the phenethyl pyrazolamine moiety anchored the hinge region and interacted with the ATP back pocket of FGFR1 in the same manner as AZD4547 (PDB ID: 4WUN)[25], but the other substituent groups oriented oppositely. Based on these observations, we designed and synthesized compound **2** with quinazoline scaffold by scaffold hopping and molecular hybridization strategies, hoping to develop a new class of FGFR inhibitors. Ethyl piperazine derived from BGJ398 was used in place of dimethyl piperazine owing to its easier accessibility and the overall synthetic convenience. Bioassay showed that **2** was highly active at the enzyme level (FGFR2 IC_{50} < 10 nM), but the cellular potency was poor (SNU16 cell proliferation inhibition IC_{50} > 100 nM), we speculated that **2** was too bulky to permeate the cell membrane and the relevant subcellular compartments. To this end, we tried to reduce the size of **2** by removing its isoxazole moiety to form compound **3** (Figure 1), because phenyl ring occupied the ATP back pocket of FGFR which was a well-established potency and selectivity handle,[19] and solubilizing groups were beneficial to optimize the physicochemical properties of TKIs. To our delight, compound **3** exhibited low nanomolar potency at both enzymatic and cellular levels against FGFR2. Thereby structural optimization of **3** was carried out to gain further insight into the potential of this series of compounds.

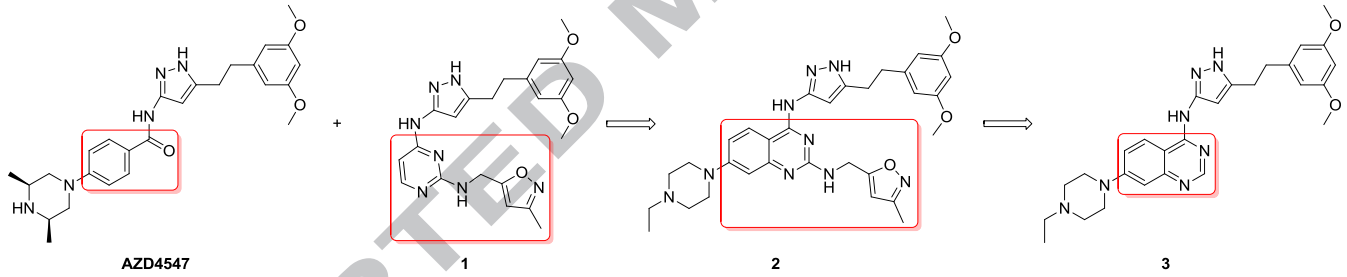
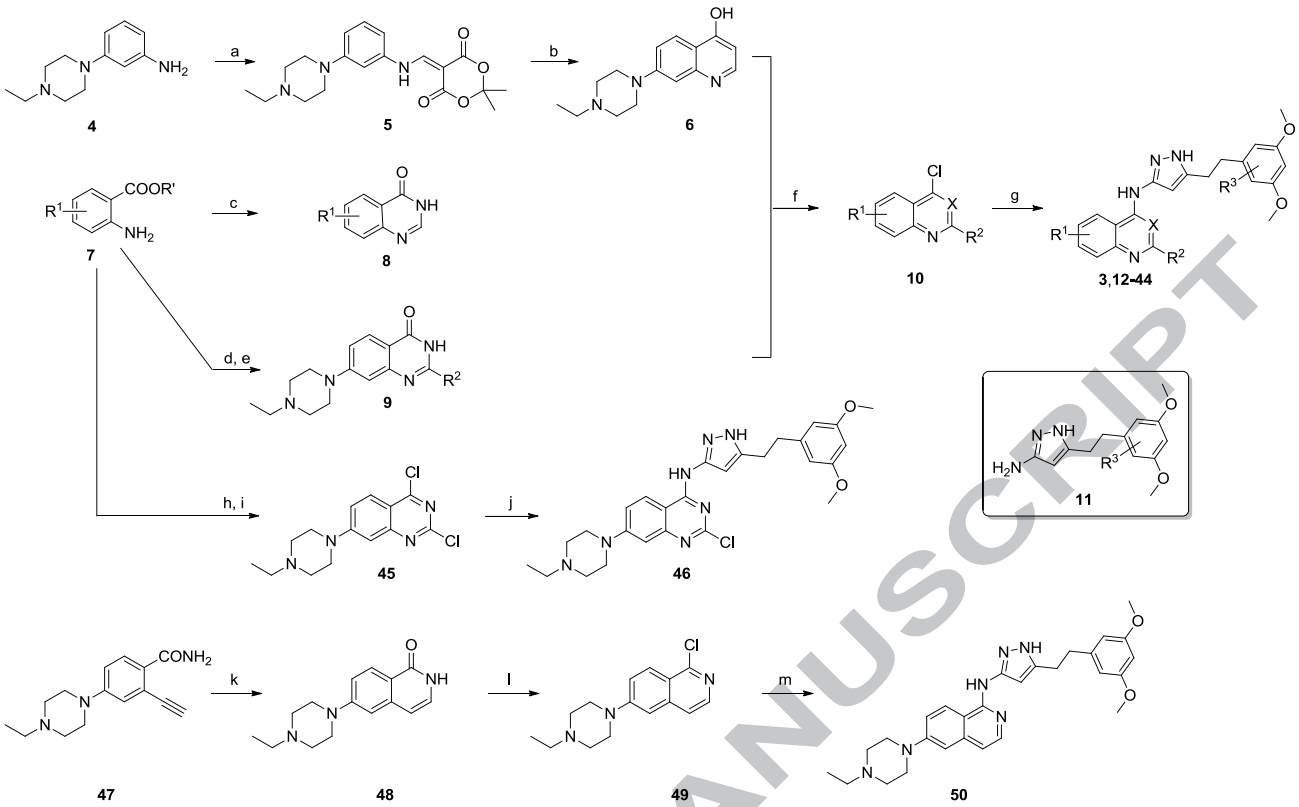


Figure 1. Design of pyrazolylaminoquinazolines.

The synthesis of compounds **3**, **12**–**44**, **46** and **50** were shown in Scheme 1 (refer to supporting information for the preparation of the starting materials). Condensation of **4** with 2,2-dimethyl-1,3-dioxane-4,6-dione and triethyl orthoformate gave intermediate **5**, which was subsequently cyclized to produce the 4-hydroxyquinoline **6**. Quinazolinones **8** were prepared by treatment of diverse anthranilic acid esters **7** with formamidine acetate. 2-Substituted quinazolinones **9** were prepared by reaction of **7** with different acyl chlorides to generate intermediate amides which then underwent cyclization with ammonium hydroxide in a pressure tube. Compounds **6**, **8**, and **9** were converted to their corresponding chlorides **10** with phosphorus oxychloride. Finally, an acid-catalyzed nucleophilic substitution of **10** with another key intermediate **11** under microwave (MW) irradiation afforded the final products **3** and **12**–**44** in good yields. Dichloro compound **45** was synthesized by a two-step sequence: cyclization of **7** with urea and subsequent chlorination with phosphorus oxychloride. Displacement of the 4-Cl in **45** was performed in a sealed tube to afford analogue **46**. $CpRuCl(PPh_3)_2$ catalyzed cyclization of **47** yielded **48**, which was then transformed to chloride **49** in refluxing $POCl_3$, nucleophilic attack of **49** by **11** under harsh microwave condition generated **50**.

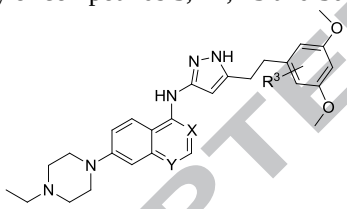
Guagnano and colleagues reported some important points about SAR of phenyl ring moiety.[19] First, the presence of two methoxy groups at the C3 and C5 positions of the phenyl ring is necessary to maintain the high affinity and selectivity for inhibitors of FGFR, so we kept the 3,5-dimethoxy-phenyl moiety unchanged in all new analogues. Second, in some rigid structures, introducing chlorine atoms in the ortho positions of the dimethoxy-phenyl ring would enhance selectivity versus VEGFR2, therefore we prepared the 2,6-dichloro compound **12** (Table 1) which was proven to be highly potent at both enzymatic and cellular levels as expected.



Scheme 1. Reagents and conditions: (a) triethyl orthoformate, 2,2-dimethyl-1,3-dioxane-4,6-dione, 90 °C, 35%; (b) diphenyl ether, 230 °C, 60%–68%; (c) formamidine acetate, 2-methoxyethanol, reflux, 80%–95%; (d) R^2COCl, CH_2Cl_2; (e) ammonium hydroxide, 120 °C, sealed tube, 70%–75% for two steps; (f) $POCl_3$, DIPEA, toluene, 90 °C, 20%–86%; (g) **11**, 1 M HCl, THF, MW, 50 °C, 80%–90%; (h) urea, 200 °C; (i) $POCl_3$, DIPEA, reflux, 58% for 2 steps; (j) **11**, AcONa, THF/H_2O, 90 °C, sealed tube, 51%; (k) $CpRuCl(PPh_3)_2$, pyridine, 90 °C, 99%; (l) $POCl_3$, reflux, 65%; (m) **11**, K_2CO_3, DMF, MW, 190 °C, 46%.

Table 1

Biological activity of compounds **3**, **12**, **13** and **50**



Compound	X	Y	R^3	IC_{50} (nM)[a]	
				FGFR2 kinase inhibition	SNU16 cells proliferation inhibition
3	N	N	H	0.8 ± 0.2	1.2 ± 0.0
12	N	N	2,6-di-Cl	0.3 ± 0.1	1.4 ± 0.3
13	CH	N	H	29.9% @ 100 nM	ND[b]
50	N	CH	H	1.2 ± 0.2	1.8 ± 0.4
AZD4547	-		-	1.2 ± 0.2	3.6 ± 1.2

[a]Values are the mean ± SD of two independent assays.

[b]ND, data not determined.

A quinoline analogue **13** and isoquinoline **50** (Table 1) were prepared to investigate the effect of the isosteric replacement. Unfortunately, the replacement of quinazoline with quinoline led to a dramatic loss of activity. Pierce reported that there may exist aromatic CH···X (X can be O or N) H-bonds in quinazoline-containing structures.[26] In our pyrazolylaminoquinazoline scaffolds, the pyrazole CH and quinazoline N-3 nitrogen may form intramolecular hydrogen bonds, which would stabilize the compound in the planar conformation to form critical hydrogen bonds with the hinge strand in the ATP binding site; in contrast, quinoline moiety did not have such N-3 nitrogen to provide such CH···N H-bonds, which could explain the low efficacy for **13**. While the N-3 nitrogen played an important role in preserving affinity, the N-1 was not indispensable, compound **50** exhibited great potency at both the enzymatic and cellular levels. Then our further SARs investigation focused on substitution on quinazoline moiety.

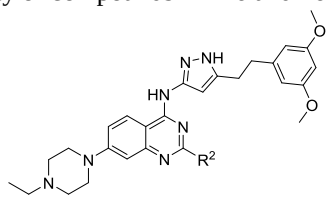
C2-substitution was first explored. Straight-chain aliphatic groups and chlorine atom were incorporated at the quinazoline C2 to maintain the molecule size smaller. The resulting compounds **14**–**16** and **46** displayed subnanomolar potency on enzyme, however they were less potent on SNU16 cells (Table 2). These data revealed that C2-substitution was detrimental to cellular activity.

The next modification was investigated on the effect of the substitution at quinazoline C5 and C8 (Table 3). Compound **18** with chlorine substitution at C5 was five-fold less potent than unsubstituted **17**, and methoxy-substitution displayed an even worse effect (**19** vs **17**), which indicated that quinazoline C5 was not tolerable to any substitution. Substitution on quinazoline C8 was also proven not to be beneficial to the activity (compounds **20**–**22**).

Then a large diversity of substituents, rigid or flexible, cyclic or linear, basic or neutral, were explored at C7 (Table 4). The piperazine substituents (**3**, **23**–**30**) generally led to more potent cellular proliferation inhibition than did other heterocyclic (**31**–**33**) or flexible substituents (**37**–**39**). Bulky benzenesulfonyl substitution (**27**) led to a degradation of potency. Eliminating the proximal piperazine nitrogen atom led to a slight but consistent reduction in potency in the cellular proliferation assay (**34** vs **23**);

the removal of the distal nitrogen dramatically deteriorated the cellular potency (**35**); replacement of the distal methylene of **35** with an oxygen retrieved cellular proliferation inhibition (**36**). Compound **40** with no polar groups retained a good level of enzyme inhibition, but the cellular potency was significantly reduced, which may be attributed to decreased cell permeability. These data suggested that substitution at C7 was well tolerated by the enzyme with compounds showing low nanomolar inhibition of FGFR2. The good tolerability of substitution at C7 could be ascribed to the fact that this moiety of the series of compounds were exposed to the solvent region, and therefore it was ideal to anchor various side chains bearing solubilizing groups to not only enhance the *in vitro* potencies but also optimize the physicochemical properties of the compounds. Translocating the C7 side chain to C6 led to significant decrease in potencies (Table 5) (**41** vs **39**, **42** vs **3**), suggesting that substitution at C6 was not tolerable.

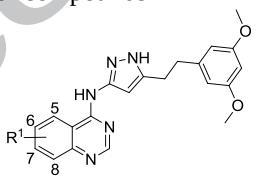
Table 2
Biological activity of compounds **14**–**16** and **46**

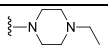


Compound	R^2	IC_{50} (nM)[a]	
		FGFR2 kinase inhibition	SNU16 cells proliferation inhibition
14	methyl	0.6 ± 0.2	105.7 ± 12.8
15	ethyl	0.5 ± 0.1	24.7 ± 0.2
16	propyl	0.7 ± 0.2	90.0 ± 30.0
46	chloro	0.6 ± 0.2	108.2 ± 36.9
AZD4547	-	1.2 ± 0.2	3.6 ± 1.2

[a]Values are the mean ± SD of two independent assays.

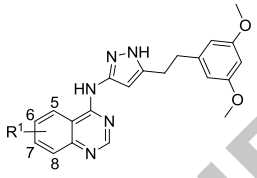
Table 3
Biological activity of compounds **17**–**22**

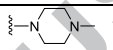
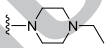
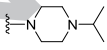
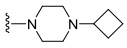
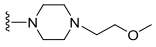
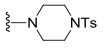
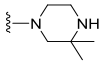
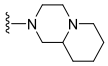
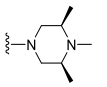
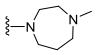
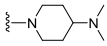
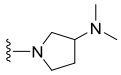
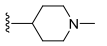
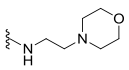
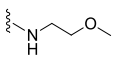
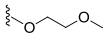


Compound	5	8	IC_{50} (nM)[a]	
			FGFR2 kinase inhibition	SNU16 cells proliferation inhibition
17	H	H	3.2 ± 0.5	> 200
18	Cl	H	16.9 ± 0.2	> 200
19	OMe	H	100.9 ± 0.2	> 200
20	H	Cl	4.8 ± 0.9	> 200
21	H	OMe	3.9 ± 0.1	> 200
22	H		4.3 ± 0.1	13.8 ± 0.2
AZD4547	-	-	1.2 ± 0.2	3.6 ± 1.2

[a]Values are the mean ± SD of two independent assays.

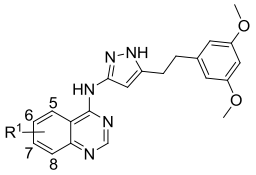
Table 4
Biological activity of compounds **3**, **23**–**40**

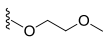
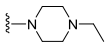
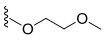
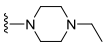


Compound	7	IC_{50} (nM)[a]	
		FGFR2 kinase inhibition	SNU16 cells proliferation inhibition
23		1.0 ± 0.2	0.7 ± 0.1
3		0.8 ± 0.2	1.2 ± 0.0
24		0.6 ± 0.0	< 0.1
25		0.6 ± 0.1	< 0.1
26		0.7 ± 0.2	49.6% @ 4 nM
27		41.8% @ 10 nM	21.2% @ 100 nM
28		0.9 ± 0.1	63.9% @ 20 nM
29		0.2 ± 0.1	0.8 ± 0.1
30		0.2 ± 0.1	< 0.1
31		0.7 ± 0.2	10.8 ± 0.6
32		0.3 ± 0.1	17.5 ± 3.6
33		0.4 ± 0.2	10.0 ± 0.6
34		0.8 ± 0.1	9.0 ± 0.5
35		87.4% @ 1 nM	36.8% @ 100 nM
36		0.4 ± 0.1	3.9 ± 0.9
37		1.3 ± 0.3	13.2 ± 1.9
38		2.7 ± 0.2	10.3 ± 1.2
39		0.9 ± 0.2	54.1 @ 20 nM
40		16.9% @ 1 nM 73.3% @ 10 nM	22.3% @ 100 nM
AZD4547	-	1.2 ± 0.2	3.6 ± 1.2

[a]Values are the mean ± SD of two independent assays.

Table 5
Biological activity of compounds **41**–**44**



Compound	7	6	IC_{50} (nM)[a]	
			FGFR2 kinase inhibition	SNU16 cells proliferation inhibition
41	H		43.9% @ 100 nM	ND[b]
42	H		29.9% @ 100 nM	ND
43			58.8% @ 100 nM	ND
44			66.7% @ 100 nM	9.7% @ 100 nM
AZD4547	-	-	1.2 ± 0.2	3.6 ± 1.2

[a]Values are the mean ± SD of two independent assays.

[b]ND, data not determined.

Five compounds with good potencies in both enzyme and cancer cells were selected for evaluation of their selectivity over VEGFR2. The result showed that they were at least 50 times more potent against FGFR2 than VEGFR2 (Table 6). Given robust enzymatic and cellular potency, as well as favorable selectivity, the representative compound **29** was selected for further evaluation.

Table 6
VEGFR2 selectivity of selected compounds

Compound	FGFR2 (IC50, nM)[a]	VEGFR2 (IC50, nM)[a]	VEGFR2/FGFR2 ratio
29	0.2 ± 0.1	21.5 ± 4.9	> 100
37	1.3 ± 0.3	> 100	> 70
39	0.9 ± 0.2	> 100	> 100
12	0.3 ± 0.1	17.5 ± 5.0	> 50
15	0.5 ± 0.1	35.8 ± 6.1	> 70
AZD4547	1.2 ± 0.2	29.5±8.6	>20

[a]Values are the mean ± SD of two independent assays.

Compound **29** was evaluated for its activity against other FGFR isoforms, including FGFR1, FGFR3, and FGFR4. Compared with its high potency against FGFR2, it showed almost similar potency against FGFR1 and FGFR3, 50-fold less potency against FGFR4, with IC_{50} values of 1.0, 5.0, 52.7 nM, respectively (Table 7), indicating that it was a potent pan-FGFR inhibitor. Further, the cellular targeting activity of compound **29** was analyzed in representative *FGFR1*-fusion protein driven human myeloid leukemia cancer cell line KG-1. As shown in Figure 2, **29** inhibited the phosphorylation of FGFR in a dose dependent manner. The phosphorylation of FGFR downstream effectors, PLCγ and ERK, was also strongly inhibited in accordance with FGFR phosphorylation. This result suggested that **29** exhibited an effective inhibition of FGFR activation and its downstream signaling. We then set to investigate the inhibitory effects of compound **29** on cell proliferation in FGFR-addicted human cancer cell lines with different mechanisms of FGFR activation. As shown in Table 8, **29** strongly inhibited the cell proliferation of these cell lines, with IC_{50} values of subnanomolar levels, confirming that **29** was a potent pan-FGFR inhibitor.

Table 7
Inhibitory activity of **29** on FGFR family kinases

Kinase	IC_{50} (nM)[a]	
	29	**AZD4547**
FGFR1	1.0 ± 0.1	0.8 ± 0.3
FGFR2	0.2 ± 0.1	1.2 ± 0.2
FGFR3	5.0 ± 1.3	5.6 ± 1.8
FGFR4	52.7 ± 0.6	45.7 ± 11.2

[a]Values are the mean ± SD of two independent assays.

Table 8
Anti-proliferative activity of **29** on FGFR addicted cell lines

Cancer cell lines	IC_{50} (nM)[a]	
	29	**AZD4547**
H1581 (*FGFR1* amplification)	< 0.5	32.9 ± 3.0
KG-1 (*FGFR1* translocation)	< 0.2	< 0.2
KATOIII (*FGFR2* amplification)	< 0.4	20.2 ± 0.5
RT112 (*FGFR3* amplification)	< 0.2	0.6 ± 0.3

[a]Values are the mean ± SD of two independent assays.

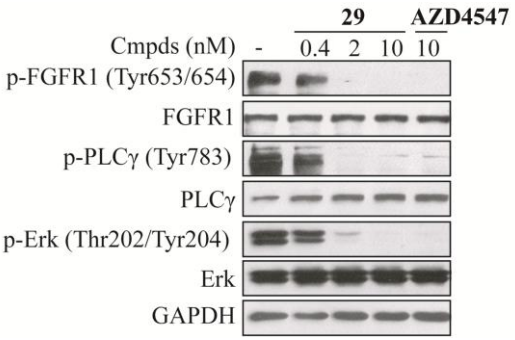


Figure 2. Compound **29** effectively inhibited the phosphorylation of FGFR1 and the downstream effectors PLCγ and Erk in KG-1cells, the specific phosphorylation sites tested of p-FGFR1, p-PLC, and p-ERK were indicated.

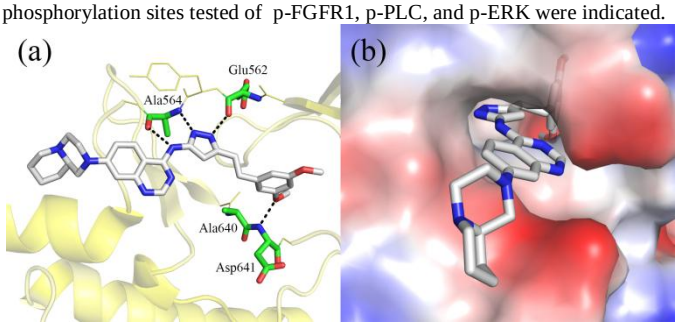


Figure 3. Compound **29** (white) was docked into the ATP binding site of a published crystallographic structure of FGFR 1 (PDB ID: 4WUN). Intermolecular hydrogen bonds between **29** and FGFR1 were shown in black dashed lines.

To clarify its binding mode, we carried out a docking study with **29** in FGFR1 kinase domain. The proposed binding mode of **29** in the FGFR1 catalytic site were illustrated in Figure 3. The pyrazolylaminoquinazoline core was in a planar conformation to form three critical H-bonds to the hinge region: the pyrazolamine NH and the adjacent pyrazole nitrogen of **29** formed two H-bonds with the carbonyl and the amino group of alanine 564, respectively; an additional H-bond occurred between the backbone carbonyl group of glutamic acid 562 and the pyrazole N-H. 3,5-Dimethoxylphenyl group penetrated into the back of ATP pocket which was thought to exert selectivity,[19] the phenyl ring was in vicinity to alanine 640 (the distance between 2'-H to the side chain of alanine 640 was approximately 4 Å), the energy penalty caused by a larger cysteine residue in VEGFR2 may offer a potential rationale for the selectivity. The 5- and 6-positions of the quinazoline were in close contact with the hinge region, accounting for the low tolerance at these positions, while 2- and 8-positions had vectors pointing into more open space, the piperazine moiety was protruded into the solvent region. The binding model was consistent with the SAR described above, which helped to rationalize the FGFR potency and selectivity.

The rapid onset of drug resistance is an important Achilles' heel for developing TKIs with long-term efficacy. The mutation of FGFR gatekeeper valine to methionine contributes to the faster autophosphorylation rate seen in V561M FGFR1. Anderson reported that AZD4547 was able to sample several binding conformations to preserve nanomolar range affinity for V561M FGFR1. The flexibility provided by the ethyl linker allows its multiple binding configurations.[27] In our modification strategy of the pyrazolylaminoquinazoline structures, the flexible ethyl linker was kept unchanged. We conducted a molecular docking study with **29** and V561M FGFR1. Superimposition of **29** with AZD4547 showed that **29** bound to each of the molecules of the asymmetric units of the V561M FGFR1 structures in different ways, which were the same as AZD4547. As both the conformations were stable for AZD4547 to exert nanomolar binding affinity in V561M FGFR1, we believed that **29** could preserve good affinity for V561M FGFR1.

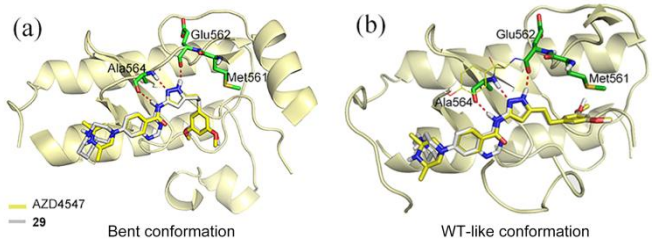


Figure 4. Compound **29** (white) was docked into the asymmetric units of the V561M FGFR1 (PDB ID: 4RWK), superimposition of **29** and AZD4547 in different binding ways were shown respectively. AZD4547 is shown in yellow, **29** in white.

In summary, we have identified pyrazolylaminoquinazoline compounds as a new class of potent and selective FGFR inhibitors. The representative compound **29** demonstrated subnanomolar inhibition against FGFR2 in both enzymatic and cellular assays, as well as exquisite selectivity over VEGFR2. Compound **29** also strongly inhibited FGFR1–3 kinase activity and suppressed FGFR signaling transduction in FGFR-addicted cancer cells. In turn, **29** potently inhibited FGFR-driven cell proliferation regardless of mechanistic complexity implicated in FGFR activation, which further confirmed that **29** was a potent pan-FGFR inhibitor. Docking study disclosed the binding mode of **29** with the FGFR1 kinase domain, which helped to rationalize the SAR study and selectivity. The flexibility of our structure offered the potential to preserve good affinity for V561M FGFR1. Further structural optimization and biological evaluation of this class of compounds are under way and will be reported in due course.

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References and notes

1. Eswarakumar, V. P.; Lax, I.; Schlessinger, J. *Cytokine Growth Factor Rev.* **2005**, *16*, 139.
2. Grose, R.; Dickson, C. *Cytokine Growth Factor Rev.* **2005**, *16*, 179.
3. Korc, M.; Friesel, R. E. *Curr. Cancer Drug Targets* **2009**, *9*, 639.
4. Knights, V.; Cook, S. J. *Pharmacol. Ther.* **2010**, *125*, 105.
5. Ahmad, I.; Iwata, T.; Leung, H. Y. *Biochim. Biophys. Acta.* **2012**, *1823*, 850.
6. Brooks, A. N.; Kilgour, E.; Smith, P. D. *Clin. Cancer Res.* **2012**, *18*, 1855.
7. Carneiro, B. A.; Meeks, J. J.; Kuzel, T. M.; Scaranti, M.; Abdulkadir, S. A.; Giles, F. J. *Cancer Treat. Rev.* **2015**, *41*, 170.
8. Saito, S.; Morishima, K.; Ui, T.; Hoshino, H.; Matsubara, D.; Ishikawa, S.; Aburatani, H.; Fukayama, M.; Hosoya, Y.; Sata, N.; Lefor, A. K.; Yasuda, Y.; Niki, T. *BMC Cancer* **2015**, *15*, doi: 10.1186/s12885-015-1065-8.
9. MacKenzie, B.; Korfei, M.; Henneke, I.; Sibinska, Z.; Tian, X.; Hezel, S.; Dilai, S.; Wasnick, R.; Schneider, B.; Wilhelm, J.; El Agha, E.; Klepetko, W.; Seeger, W.; Schermuly, R.; Günther, A.; Bellusci, S. *Respir. Res.* **2015**, *16*, doi: 10.1186/s12931-015-0242-2.
10. Zhao, D.; Lu, Y.; Yang, C.; Zhou, X.; Xu, Z. *Tumor Biol.* **2015**, *36*, 3637.
11. Inokuchi, M.; Fujimori, Y.; Otsuki, S.; Sato, Y.; Nakagawa, M.; Kojima, K. *Gastroenterol. Res. Pract.* **2015**, *2015*, doi: 10.1155/2015/796380.
12. Kumar, S. B.; Narasu, L.; Gundla, R.; Dayam, R.; Sarma, J. A. R. P. *Curr. Pharm. Des.* **2013**, *19*, 687.
13. Bello, E.; Colella, G.; Scarlato, V.; Oliva, P.; Berndt, A.; Valbusa, G.; Serra, S. C.; D'Incalci, M.; Cavalletti, E.; Giavazzi, R.; Damia, G.; Camboni, G. *Cancer Res.* **2011**, *71*, 1396.
14. Porta, C.; Giglione, P.; Liguigli, W.; Paglino, C. *Future Oncol.* **2015**, *11*, 39.
15. Renhowe, P. A.; Pecchi, S.; Shafer, C. M.; Machajewski, T. D.; Jazan, E. M.; Taylor, C.; Antonios-McCrea, W.; McBride, C. M.; Frazier, K.; Wiesmann, M.; Lapointe, G. R.; Feucht, P. H.; Warne, R. L.; Heise, C. C.; Menezes, D.; Aardalen, K.; Ye, H.; He, M.; Le, V.; Vora, J.; Jansen, J. M.; Wernette-Hammond, M. E.; Harris, A. L. *J. Med. Chem.* **2009**, *52*, 278.
16. Borzilleri, R. M.; Zheng, X.; Qian, L.; Ellis, C.; Cai, Z. W.; Wautlet, B. S.; Mortillo, S.; Jeyaseelan, R., Sr.; Kukral, D. W.; Fura, A.; Kamath, A.; Vyas, V.; Tokarski, J. S.; Barrish, J. C.; Hunt, J. T.; Lombardo, L. J.; Fargnoli, J.; Bhide, R. S. *J. Med. Chem.* **2005**, *48*, 3991.
17. Cai, Z. W.; Zhang, Y.; Borzilleri, R. M.; Qian, L.; Barbosa, S.; Wei, D.; Zheng, X.; Wu, L.; Fan, J.; Shi, Z.; Wautlet, B. S.; Mortillo, S.; Jeyaseelan, R., Sr.; Kukral, D. W.; Kamath, A.; Marathe, P.; D'Arienzo, C.; Derbin, G.; Barrish, J. C.; Robl, J. A.; Hunt, J. T.; Lombardo, L. J.; Fargnoli, J.; Bhide, R. S. *J. Med. Chem.* **2008**, *51*, 1976.
18. Gozgit, J. M.; Wong, M. J.; Moran, L.; Wardwell, S.; Mohemmad, Q. K.; Narasimhan, N. I.; Shakespeare, W. C.; Wang, F.; Clackson, T.; Rivera, V. M. *Mol. Cancer Ther.* **2012**, *11*, 690.
19. Guagnano, V.; Furet, P.; Spanka, C.; Bordas, V.; Le Douget, M.; Stamm, C.; Brueggen, J.; Jensen, M. R.; Schnell, C.; Schmid, H.; Wartmann, M.; Berghausen, J.; Drueckes, P.; Zimmerlin, A.;

Bussiere, D.; Murray, J.; Graus Porta, D. *J. Med. Chem.* **2011**, *54*, 7066.
20. Gavine, P. R.; Mooney, L.; Kilgour, E.; Thomas, A. P.; Al-Kadhimi, K.; Beck, S.; Rooney, C.; Coleman, T.; Baker, D.; Mellor, M. J.; Brooks, A. N.; Klinowska, T. *Cancer Res.* **2012**, *72*, 2045.
21. Nakanishi, Y.; Akiyama, N.; Tsukaguchi, T.; Fujii, T.; Sakata, K.; Sase, H.; Isobe, T.; Morikami, K.; Shindoh, H.; Mio, T.; Ebiike, H.; Taka, N.; Aoki, Y.; Ishii, N. *Mol. Cancer Ther.* **2014**, *13*, 2547.
22. Zhao, G.; Li, W. Y.; Chen, D.; Henry, J. R.; Li, H. Y.; Chen, Z.; Zia-Ebrahimi, M.; Bloem, L.; Zhai, Y.; Huss, K.; Peng, S. B.; McCann, D. J. *Mol. Cancer Ther.* **2011**, *10*, 2200.
23. Norman, R. A.; Schott, A. K.; Andrews, D. M.; Breed, J.; Foote, K. M.; Garner, A. P.; Ogg, D.; Orme, J. P.; Pink, J. H.; Roberts, K.; Rudge, D. A.; Thomas, A. P.; Leach, A. G. *J. Med. Chem.* **2012**, *55*, 5003.
24. Klein, T; Tucker, J; Holdgate G. A.; Norman, R. A.; Breeze, A. L. *ACS Med. Chem. Lett.* **2013**, *5*, 166.
25. Yosaatmadja, Y.; Patterson, A. V.; Smaill, J. B.; Squire, C. J. *Acta Crystallogr., Sect. D* **2015**, *71*, 525.
26. Pierce, A. C.; ter Haar, E.; Binch, H. M.; Kay, D. P.; Patel, S. R.; Li, P. *J. Med. Chem.* **2005**, *48*, 1278.
27. Sohl, C. D.; Ryan, M. R.; Luo, B.; Frey, K. M.; Anderson, K. S. *ACS Chem. Biol.* **2015**, *10*, 1319.