Bioorganic & Medicinal Chemistry Letters 20 (2010) 6067–6071

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Substituted 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines as multi-targeted inhibitors of insulin-like growth factor-1 receptor (IGF1R) and members of ErbB-family receptor kinases

Gary T. Wang *, Robert A. Mantei, Robert D. Hubbard, Julie L. Wilsbacher, Qian Zhang, Lora Tucker, Xiaoming Hu, Peter Kovar, Eric F. Johnson, Donald J. Osterling, Jennifer Bouska, Jieyi Wang, Steven K. Davidsen, Randy L. Bell, George S. Sheppard

Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA

ARTICLE INFO

Article history:
Received 30 June 2010
Revised 9 August 2010
Accepted 11 August 2010
Available online 13 August 2010

Keywords:
Multi-targeted kinase inhibitors
IGF1R
EGFR
ErbB2

ABSTRACT

This Letter describes the lead discovery, optimization, and biological characterization of a series of substituted 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines as potent inhibitors of IGF1R, EGFR, and ErbB2. The leading compound **11** showed an IGF1R IC_{50} of 12 nM, an EGFR (L858R) IC_{50} of 31 nM, and an ErbB2 IC_{50} of 11 nM, potent activity in cellular functional and anti-proliferation assays, as well as activity in an in vivo pharmacodynamic assay.

Insulin-like growth factor-1 receptor (IGF1R) and members of the ErbB family (e.g., EGFR, ErbB2) are receptor tyrosine kinases that play significant roles in cancer and have been pursued as drug targets for cancer therapy.[1,2] Several inhibitors of the ErbB-family signaling have gained regulatory approval for treatment of various cancers, including trastuzumab (Herceptin™), gefitinib (Iressa™), erlotinib (Tarceva™), and lapatinib (Tykerb™), while several inhibitors of IGF1R are currently undergoing human clinical trials as anti-cancer agents.[3] There is substantial evidence for the existence of reciprocal cross-talk between the IGF1R axis and ErbB axes of cellular signaling. Thus, acquired resistance to the EGFR inhibitors gefitinib and erlotinib or the ErbB2 inhibitor trastuzumab has been linked to activation of IGF1R signaling through induced overexpression of IGF1R or loss of IGF binding proteins.[4–6] Conversely, resistance to the IGF1R inhibitor BMS-536924 has been linked to ErbB2 signaling.[7] Additionally, synergy between IGF1R inhibition and ErbB inhibition has been demonstrated both in vitro in anti-proliferation assays and in vivo in xenograft models using small molecules, antibodies, and siRNA.[8–11] These literature data strongly suggest that molecules targeting both IGF1R and ErbB-family members may offer superior anti-tumor efficacy and delayed onset of resistance in comparison with agents that target either IGF1R or ErbB-family members alone.

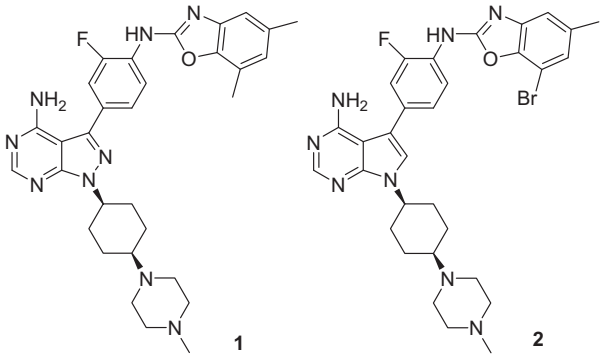
Recently, we have disclosed a series of inhibitors targeting both IGF1R and ErbB-family members based on 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines bearing 1*H*-benzo[*d*]imidazol-5-yl moieties at the C3 position.[12] These compounds were derived from high throughput screening hits.[13] Also uncovered in this high throughput screen were 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine and 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine bearing 4-(benzo[*d*]oxazol-2-ylamino)phenyl moieties, compounds **1** and **2** (Table 1). These molecules exhibited modest activity against IGF1R and weak activity against EGFR and ErbB2. Molecular modeling suggested that the 4-amino-pyrazolopyrimidine would form the key H-bonding interaction with the hinge residues of the target kinases, while the benzoxazole head group would occupy the hydrophobic back pocket of the typical DFG-out conformation of these kinases and the cyclohexyl-piperazine tail group would extend toward the solvent front. Herein, we describe our efforts to optimize these leads. Our goal was to simultaneously improve the potency against IGF1R, EGFR and/or ErbB2, while improving drug-like properties in order to identify candidates suitable for clinical development.

As depicted in Scheme 1, the synthesis of the target molecules started with the condensation of 4-bromo-isothiocyanates **3** with *ortho*-hydroxy-anilines **4** to afford the 4-(benzo[*d*]oxazol-2-ylamino)phenyl bromides **5** in good yield. These bromides were converted to the corresponding pinacol boronates **6** under the standard conditions. Reductive amination of the previously

* Corresponding author. Tel.: +1 847 937 2489; fax: +1 847 937 8378.
E-mail address: gary.t.wang@abbott.com (G.T. Wang).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.08.052

Table 1
Pyrazolopyrimidine and pyrrolopyrimidine screening hits



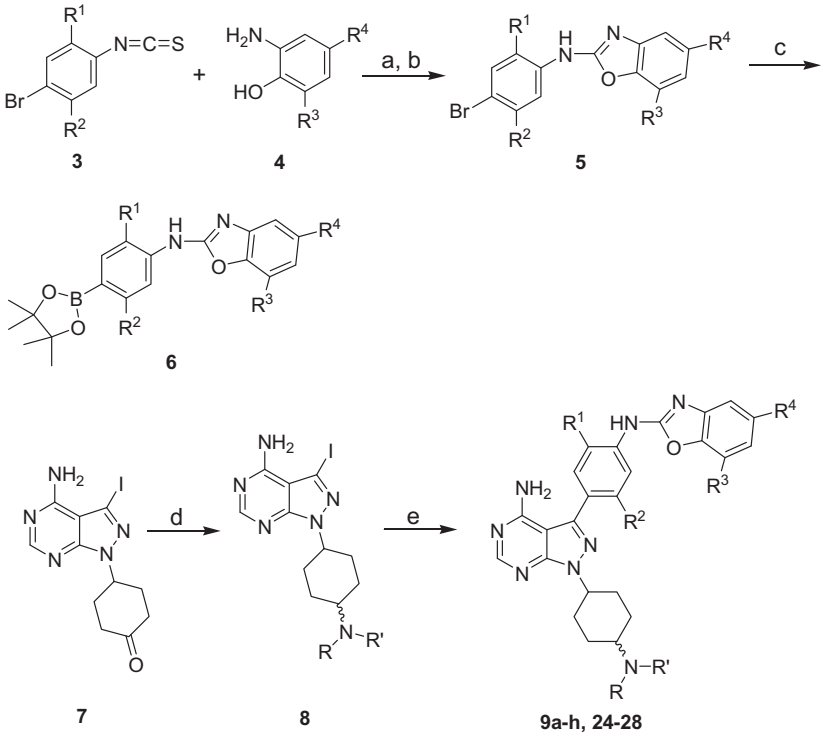
Compd	Enzyme IC_{50} (nM)[15]		
	IGF1R	EGFR (L858R)	ErbB2
1	160	2270	4030
2	94	1450	>62,500

described ketone **7** gave a mixture of *cis*/*trans* isomers of **8** in approximately 1:1 ratio,[14] which can be separated by column chromatography or HPLC. Suzuki coupling of **8** with boronates **6** under microwave conditions gave the target compounds in fair to good yields.

We first focused our attention on the structure–activity relationships (SAR) of the substitution on the benzoxazole moiety by holding the N1-substitution of the pyrazolopyrimidine core constant (Table 2). With reference to compound **9a** which has an unsubstituted benzoxazole head group, introduction of cyano (**9b**) or carbomethoxy (**9c**) at the C7-position of the benzoxazole moiety resulted in modest improvement in potency against IGF1R but decrease in potency against EGFR. However, a chloro-substitution at this position (**9d**) gave rise to over 10-fold enhancement in IGF1R potency and major improvement in EGFR potency. The ErbB2 potency of **9d** also showed major improvement in comparison with compounds **1** and **2**. Computer modeling of both IGF1R and EGFR with compound **9d** did not provide conclusive rationale for this observation. Moving the chlorine substitution from C7 (**9e**) to C5 (**9f**) of benzoxazole resulted in loss of potencies. Similarly, substitution of the benzoxazole at both C7 and C5 positions (**9g** and **9h**) also reduced potency against all three target enzymes. Thus, 7-chloro-benzoxazole was found to be optimal based on this study.

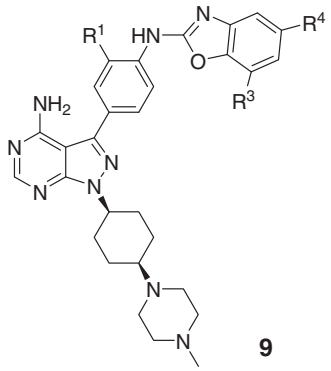
With the preferred benzoxazole substituent identified, we expended a significant effort optimizing the tail groups at the N1-position of the pyrazolopyrimidine core. A variety of linking moieties and polar capping groups were examined, but saturated six-membered ring systems were found to be preferred (data not shown). Synthesis of this sub-class of compounds is described in Schemes 2 and 3. Suzuki coupling of **7** with 7-chloro-*N*-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)benzo[*d*]oxazol-2-amine (**6**, $R_1 = R_2 = R_4 = H$, $R_3 = Cl$) gave ketone **10**. Reductive amination of **10** gave separable *cis* and *trans* amines, including compounds **11**–**17**. Alternatively, compound **20** was obtained via Mitsunobu reaction of **18** with *t*-butyl 4-hydroxy-piperidine-1-carboxylate, followed by Suzuki coupling and removal of the Boc-protecting group. Reductive alkylation of **20** furnished compounds **21**–**23**.

The enzyme inhibitory activities of compounds **11**–**17** and **21**–**23** are summarized in Table 3. In comparison with compound **9d** (Table 2), simply changing the substitution geometry of the cyclohexyl spacer from *cis* (**9d**) to *trans* (**11**) resulted in sixfold improvement in IGF1R potency, 12-fold improvement in EGFR potency and 26-fold improvement in ErbB2 potency. This strong preference for *trans* geometry was also evident from the comparison of **13** with **14** and **15** with **16**. Changing the methyl substituent on the terminal piperazine nitrogen of **11** to other groups generally



Scheme 1. Reagents and conditions: (a) THF, rt, overnight; (b) LiOH, H_2O_2, 50–80%; (c) bis(pinacolato)diboron, $Pd(dppf)_2$, dppf, KOAc, dioxane, 80 °C, 40–70%; (d) RR′NH, HOAc, CH_2Cl_2, $NaBH(OAc)_3$, 50–75%; (e) **6**, $Pd(PPh_3)_4$, C_5F, DME, MeOH, 150 °C, microwave, 40–75%.

Table 2
SAR of benzoxazole substitution



9

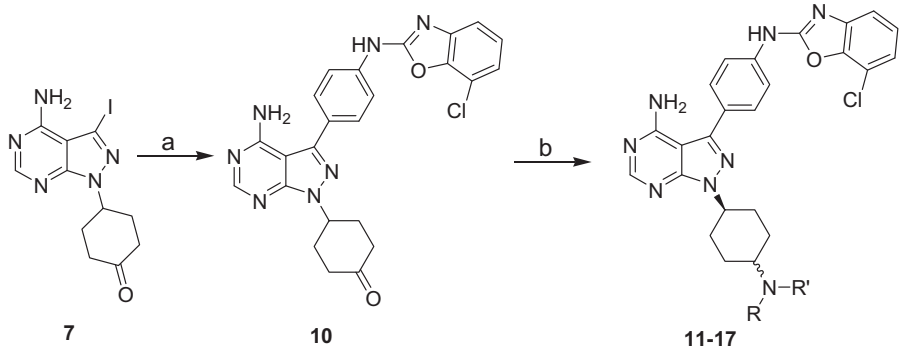
Compd	R^1	R^3	R^4	Enzyme IC_{50} (nM)[15]		
				IGF1R	EGFR (L858R)	ErbB2
9a	H	H	H	810	1660	NT
9b	H	–CN	H	113	2810	NT
9c	H	$–CO_2Me$	H	179	3590	NT
9d	H	Cl	H	77	393	200
9e	F	Cl	H	72	852	1350
9f	F	H	Cl	415	1470	NT
9g	H	Cl	CH_3	348	1130	5230
9h	H	Cl	Cl	584	639	NT

NT: not tested.

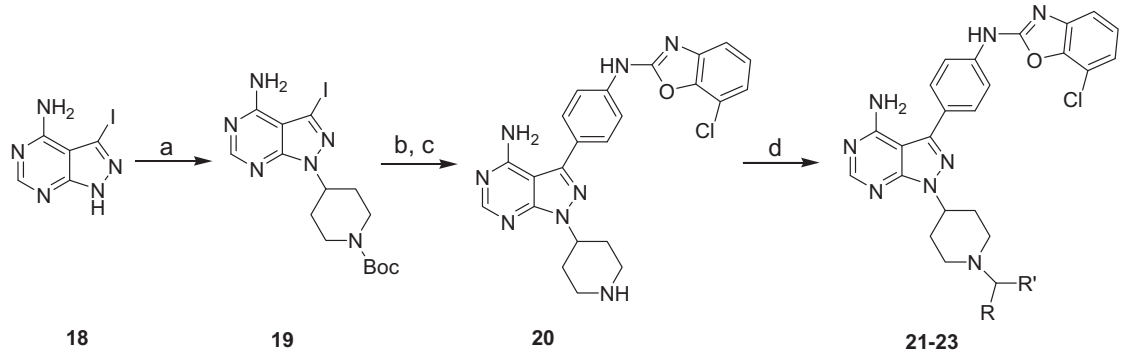
resulted in reduced potency (**13**, **15**, **17**). Replacing the cyclohexyl spacer with a piperidine moiety also led to decrease in potency (**21–23**), particularly against on IGF1R and EGFR potencies.

We briefly investigated the SAR of substitution on the linking phenyl ring (Table 4). A fluorine placed on this phenyl ring *ortho* to the 2-amino-benzoxazole head group gave compound **24** that was essentially equipotent to the parent compound **11** against all three target enzymes. A chlorine group placed on this phenyl ring at the position *ortho* to the 2-amino-benzoxazole head group (**25**) reduced the activity against IGF1R fourfold, whereas compound **27** with the Cl *ortho* to the pyrazolopyrimidine core was equipotent to **11** against IGF1R. The potency of **25** and **27** against EGFR and ErbB2 was reduced by 2- to 4-fold relative to **11**. Substituting the linking phenyl ring with a methyl group (**26** and **28**) resulted in greater loss of activities against all three target enzymes compared to parent analog **11**.

In addition to measuring the kinase inhibition activities, selected compounds were screened in functional cellular assays for their ability to inhibit the cellular phosphorylation of the target receptors. The assays were carried out in ELISA format using MiaPaCa cells, a human pancreatic carcinoma cell line which expresses both IGF1R and EGFR, and BT474, a human breast tumor cell line which highly expresses ErbB2. The cellular activities of compounds **11** and **24** are shown in Table 5. Compound **11** and close analog **24** showed potent inhibition of IGF1R in MiaPaCa cells and ErbB2 phosphorylation in BT474 cells, but were about 10-fold less potent in the inhibition of EGFR phosphorylation. The poor potency of the inhibition of EGFR phosphorylation (EC_{50} >1 μM) was consistent for all the analogs tested in this series. Since MiaPaCa cells express the wild-type EGFR, whereas our EGFR enzyme screening assay was carried out employing EGFR harboring the L858R activating mutation, we determined the IC_{50} of compound **11** against the wild-type EGFR, to be 2 nM versus 31 nM against EGFR L858R (Table 3). This result seemingly ruled out the possibility that differ-

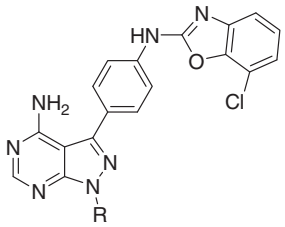


Scheme 2. Reagents and conditions: (a) **6** (R^1 = R^2 = R^4 = H, R^3 = Cl), $Pd(PPh_3)_4$, C_5F, DME, MeOH, 150 °C, microwave, 56%; (b) RR′NH, HOAc, CH_2Cl_2, $NaBH(OAc)_3$, 30–40%.



Scheme 3. Reagents and conditions: (a) *t*-Butyl 4-hydroxy-piperidine-1-carboxylate, Ph_3P, DIAD, THF, 58%; (b) **6** (R^1 = R^2 = R^4 = H, R^3 = Cl), $Pd(PPh_3)_4$, C_5F, DME, MeOH, 150 °C, microwave, 73%; (c) TFA, CH_2Cl_2, ~95%; (d) RR′C(O), HOAc, CH_2Cl_2, $NaBH(OAc)_3$, 41–90%.

Table 3
SAR of the amine tail group



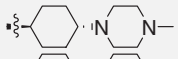
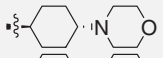
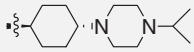
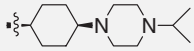
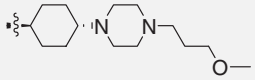
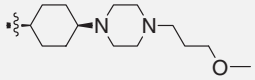
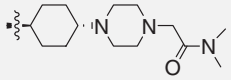
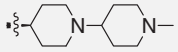
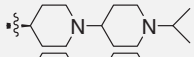
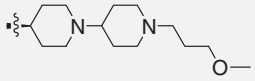
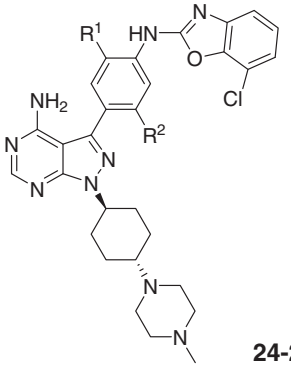
Compd	R	Enzyme IC_{50} (nM)[15]		
		IGF1R	EGFR (L858R)	ErbB2
11		12.5	31.5	7.5
12		42.0	1020	334
13		45.1	53.7	9.8
14		174	322	62
15		72.4	108	21.5
16		424	874	397
17		126	1840	21.5
21		108	99	14.5
22		115	97	15.1
23		283	264	13.8

Table 4
SAR of the substitution on the linking phenyl ring



24-28

Compd	R^1	R^2	Enzyme IC_{50} (nM)		
			IGF1R	EGFR (L858R)	ErbB2
24	F	H	7.5	37	16
25	Cl	H	49	63	29
26	CH_3	H	196	156	NT
27	H	Cl	4.1	86	15.4
28	H	CH_3	22	530	478

ence in the sensitivity of different EGFR constructs to this class of compounds could account for the observed lack of potency in EGFR phosphorylation inhibition. For comparison, the functional cellular

Table 5
Cellular activities of selected compounds

Compd	Inhibition of receptor phosphorylation IC_{50} (nM)[a]		
	pIGF1R	pEGFR	pErbB2
11	207	2300	340
24	87	2880	362
Erlotinib	>30,000	51	6730
Lapatinib	>30,000	433	140
PQIP	65	>30,000	>30,000

[a] ELISA assays of receptor auto-phosphorylation, in MiaPaCa cells for pIGF1R and pEGFR and BT474 cells for pErbB2.

activities of the known EGFR inhibitor erlotinib,[16] EGFR/ErbB2 inhibitor lapatinib,[16] and IGF1R inhibitor PQIP[17] were also measured using the same cell lines (Table 5).

Compound **11** was chosen for further characterization. The pharmacokinetic properties of **11** are summarized in Table 6. In the rat, this compound showed a relatively long $t_{1/2}$ when dosed intravenously (iv) as well as a late t_{max} when dosed orally, but the oral C_{max} was low and the oral bioavailability was a modest 30%. In mouse, the clearance further increased, both the iv $t_{1/2}$ and the oral t_{max} significantly decreased, and the oral bioavailability decreased to 20%.

Compound **11** was also evaluated in an in vivo pharmacodynamic (PD) model.[18] In this study, female C57BL/6 mice were dosed with the test compounds or vehicle control. One hour later, the mice were given IGF-1 intravenously (0.5 mg/kg). The animals were sacrificed 10 min later, and drug levels in plasma and other tissues was determined. The IGF1R phosphorylation status in the homogenized lung tissue was analyzed by Western blot, along with the phosphorylated Akt (p-Akt) status. As shown in Figure 1, intravenous dosing of compound **11** resulted ~50% inhibition of pIGF1R at 30 mg/kg and nearly complete inhibition at 60 mg/kg. Significant inhibition of p-Akt was also observed.

Table 6
Pharmacokinetic data of compound **11**

	Units	Mouse	Rat
IV DNAUC[a]	μg-h/mL/mg/kg	0.94	1.26
IV $t_{1/2}$	h	2.4	8.4
IV CLp	L/h/kg	1.91	0.91
PO DNAUC[a]	μg-h/mL/mg/kg	0.19	0.38
PO t_{max}	h	3.0	8.0
PO C_{max}	μM	0.17[b]	0.16[c]
F	%	20	30

[a] Dose-normalized area under the curve.
[b] Dosed at 10 mpk.
[c] Dosed at 5 mpk.

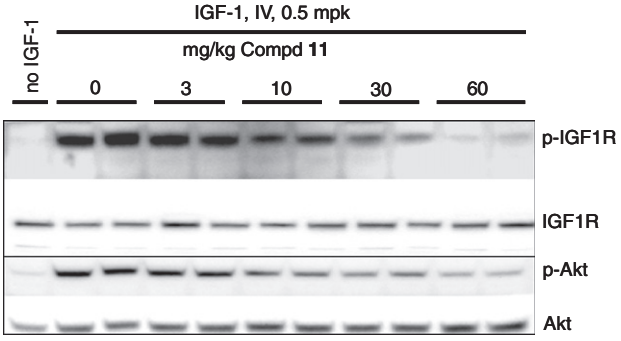


Figure 1. In vivo pharmacodynamic assay: inhibition of IGF1R and Akt phosphorylation by compound **11** in C57BL/6 mice.

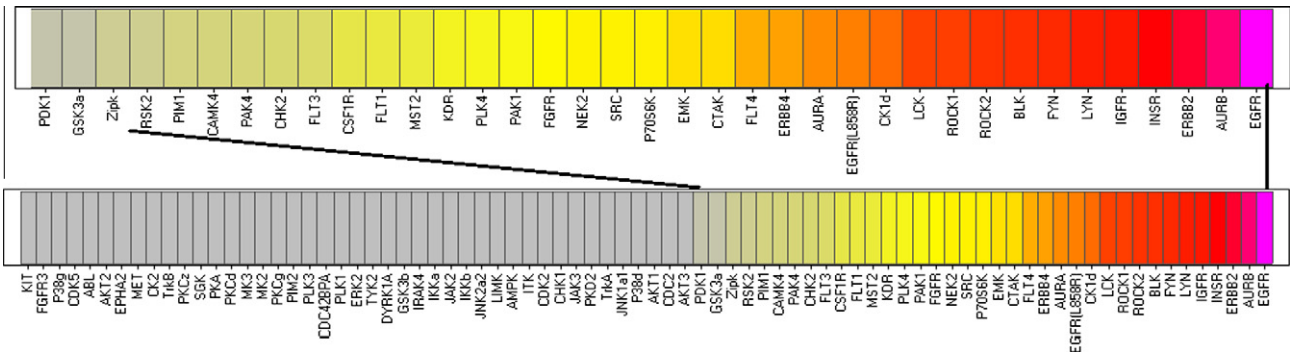


Figure 2. Heat map showing the inhibitory activity of compound **11** against 80 kinases. Pink: IC_{50} <10 nM; Red: IC_{50} 10–100 nM; Yellow: IC_{50} 100–1000 nM; Gray: IC_{50} >1000 nM.

The kinase selectivity of compound **11** was assessed against a panel of 80 kinases and the results are shown in a heat map (Fig. 2). Besides the intended receptor tyrosine kinases (IGF1R, IR, EGFR, ErbB2), compound **11** also potently inhibits several of the Src family of non-receptor tyrosine kinases including Fyn, Lyn, and Lck. This was not surprising in consideration of literature reports on related compounds as Src family inhibitors.[19] Unexpectedly, the compound also inhibits the Aurora family of serine–threonine kinases, being particularly potent against Aurora B.

In summary, we have identified a series of substituted 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines as potent inhibitors of IGF1R, EGFR, and ErbB2 receptor kinases. Many analogs showed potent and balanced activity against all three target enzymes, as exemplified by the leading compound **11** (IGF1R IC_{50} of 12 nM, EGFR (L858R) IC_{50} of 31 nM, and ErbB2 IC_{50} of 11 nM). In cellular functional assays, these compounds potently inhibit the phosphorylation of IGF1R and ErbB2, but not EGFR (EC_{50} >1 μM). Compound **11** also showed activity in an in vivo pharmacodynamic assay of IGF1R inhibition.

References and notes

1. (a) Pollak, M. *Nat. Rev. Cancer* **2008**, *8*, 915; (b) Sachdev, D.; Yee, D. *Mol. Cancer Ther.* **2007**, *6*, 1.
2. Hynes, N.; Lane, H. *Nat. Rev. Cancer* **2005**, *5*, 341.
3. Li, R.; Pourpak, A.; Morris, S. W. *J. Med. Chem.* **2009**, *52*, 4981.
4. Jones, H. E.; Goddard, L.; Gee, J. M. W.; Hiscox, S.; Rubini, M.; Barrow, D.; Knowlden, J. M.; Williams, S.; Walkeling, A. E.; Nicholson, R. I. *Endocr. Relat. Cancer* **2004**, *11*, 793.
5. Nahta, R.; Yuan, L. Y.; Du, Y.; Esteva, F. J. *Mol. Cancer Ther.* **2007**, *6*, 667.
6. Guix, M.; Faber, A. C.; Wang, S. E.; Olivares, M. G.; Song, Y.; Qu, S.; Rinehart, C. R.; Seidel, B.; Yee, D.; Arteaga, C. L.; Engelman, J. A. *J. Clin. Invest.* **2008**, *118*, 2609.
7. Haluska, P.; Carboni, J. M.; TenEyck, C.; Attar, R. M.; Hou, X.; Yu, C.; Sagar, M.; Wong, T. W.; Gottardis, M. M.; Erlichman, C. *Mol. Cancer Ther.* **2008**, *7*, 2589.
8. Wilsbacher, J. L.; Zhang, Q.; Tucker, L. A.; Hubbard, R. D.; Sheppard, G. S.; Bamaung, N. Y.; Fidanze, S. D.; Wang, G. T.; Hu, X.; Davidsen, S. K.; Bell, R. L.; Wang, J. *J. Biol. Chem.* **2008**, *283*, 23712.
9. Charkraborty, A. K.; Liang, K.; DiGiovanna, M. P. *Cancer Res.* **2008**, *68*, 1538.
10. Lu, D.; Zhang, H.; Koo, H.; Tonra, J.; Balderes, P.; Prewett, M.; Corcoran, E.; Mangalampalli, V.; Bassi, R.; Anselma, D.; Patel, D.; Kang, X.; Ludwig, D. L.; Hicklin, D. J.; Witte, L.; Zhu, Z. *J. Biol. Chem.* **2005**, *280*, 19665.
11. Goetsch, L.; Gonzalez, A.; Leger, O.; Beck, A.; Pauwels, P. J.; Haeuw, J. F.; Corvaia, N. *Intl. J. Cancer* **2005**, *113*, 316.
12. Hubbard, R. D.; Bamaung, N. Y.; Fidanze, S. D.; Erickson, S. A.; Palazzo, F.; Wilsbacher, J. L.; Zhang, Q.; Tucker, L. A.; Hu, X.; Kovar, P.; Osterling, D. J.; Johnson, E. F.; Bouska, J.; Wang, J.; Davidsen, S. K.; Bell, R. L.; Sheppard, G. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1718.
13. Hubbard, R. D.; Bamaung, N. Y.; Palazzo, F.; Zhang, Q.; Kovar, P.; Osterling, D. J.; Hu, X.; Wilsbacher, J. L.; Johnson, E. F.; Bouska, J.; Wang, J.; Bell, R. L.; Davidsen, S. K.; Sheppard, G. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5406.
14. (a) Hirst, G. C.; Calderwood, D.; Wishart, N.; Rafferty, P.; Ritter, K.; Arnold, L. D.; Friedman, M. M. WO 01/19829.; (b) Borhan, D.; Calderwood, D.; Dixon, R. W.; Hirst, G. C.; Hrnciar, P.; Loew, A.; Leung, A.; Ritter, K. WO 03/020880.
15. The kinase inhibitory activities of the test compounds were measured using a homogeneous time-resolved fluorescence assay, as described in Ref. 12.
16. Lackey, K. *Curr. Top. Med. Chem.* **2006**, *6*, 435.
17. Ji, Q.; Mulvihill, M. J.; Rosenfeld-Franklin, M.; Cooke, A.; Feng, L.; Mak, G.; O'Connor, M.; Yao, Y.; Pirritt, C.; Buck, E.; Eyzaguirre, A.; Arnold, L. D.; Gibson, N. W.; Pachter, J. A. *Mol. Cancer Ther.* **2007**, *6*, 2158.
18. Garcia-Echeverria, C.; Pearson, M. A.; Marti, A.; Meyer, T.; Mestan, J.; Zimmermann, J.; Gao, J.; Brueggen, J.; Capraro, H.-G.; Cozens, R.; Evans, D. B.; Fabbro, D.; Furet, P.; Porta, D. G.; Liebetanz, J.; Martiny-Baron, G.; Reutz, S.; Hofmann, F. *Cancer Cell* **2004**, *5*, 231.
19. (a) Burchat, A.; Borhani, D. W.; Calderwood, D. J.; Hirst, G. C.; Li, B.; Stachlewitz, R. F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 118; (b) Bohani, D. W.; Calderwood, D. J.; Friedman, M. M.; Hirst, G. C.; Li, B.; Leung, K. W.; McRae, B.; Ratnofsky, S.; Ritter, K.; Waegell, W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2613.