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Synthesis and evaluation of novel pyrimidine-based dual EGFR/Her-2 inhibitors

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ABSTRACT

A structure–activity relationship study of 4-anilinopyrimidines for dual EGFR/Her-2 inhibitor has resulted in the identification of 4-anilino-5-alkenyl or 5-alkynyl-6-methylpyrimidine derivatives that have exhibited effective inhibitory activity against both enzymes. The presence of 5-alkenyl or 5-alkynyl moiety bearing terminal hydrophilic group played important role for inhibition of these enzymes. Selected compounds in the series demonstrated some activity against Her-2 dependent cell line (BT474).

Receptor tyrosine kinases, critical components of cellular signal transduction, are involved in many diseases, particularly cancer.[1] Epidermal growth factor receptor (EGFR/ErbB-1) and Her-2(ErbB-2), members of the Type 1 receptor tyrosine kinase family, play a key role in cell proliferation and differentiation.[2] Overexpression of these receptors is found in a variety of cancers such as breast, ovarian, colon and prostate cancers, and has been associated with poor prognosis in patients.[3] The ErbB receptors can be activated through homo or heterodimerization with other receptors resulting in phosphorylation events and downstream signaling that produces excessive growth by inducing cell proliferation and inhibiting apoptotic pathways.[2] Therefore, simultaneous inhibition of EGFR and Her-2 has been expected to provide better efficacy than single receptor targeting. Lapatinib ditosylate (Tykerb, **1**) (Fig. 1), an oral dual EGFR and Her-2 inhibitor, was recently been approved for treatment of patients with Her-2 over-expressing advanced or metastatic breast cancer.[4] This drug has effective dual EGFR/Her-2 inhibitory activity and high selectivity against other kinases,[5] and shows good therapeutic efficacy in clinical practice.

Among known kinase inhibitors, the 4-anilinoqunaizoline scaffold is the most common template for inhibitors of the ErbB family. The quinazoline moiety fits into the ATP-binding pocket in the kinase domain, while the aniline ring fills an adjacent lipophilic pocket.[6] However, the lipophilic character of the 4-anilinoquinazoline moiety may cause their poor aqueous solubility, which influences their ADME aspect, and has posed a variety of challenges for their drug development.[7] Therefore, intense research has recently been devoted to exploring alternatives to the quinazoline moiety, and other fused heteroaromatic derivatives, such as pyrrolotriazine thienopyrimidine and thiazolopyrimidine[8] were reported as dual EGFR/Her-2 inhibitor. However, monoheteroaromatic derivatives which display effective antiproliferative activities against tumor cell lines have been limited.[9]

In our effort to discover and develop non-quinazoline derivatives for effective EGFR/Her-2 dual inhibitors as antitumor agents, we focused on the pyrimidine scaffold, which could mimic the function of the quinazoline moiety and could be expected to offer better physicochemical properties. In this Letter, we will report an SAR study of pyrimidine derivatives for EGFR/Her-2 dual inhibitors.

Lapatinib (**1**), Gefitinib (**2**)[10] and Erlotinib (**3**)[11] are ATP-competitive EGFR inhibitors that contain a 4-anilinoquinazoline and a linker at the C-6 position with the terminal hydrophilic part as a common structural feature. However, only Lapatinib displays effective potency against Her-2, with 100-fold greater activity than that of either Gefitinib or Erlotinib.[12] The differences in Her-2 inhibitory potency suggested that the large 4-benzyloxy aniline moiety present in Lapatinib plays an important role for the inhibition of Her-2. From these findings, we assumed that the large aniline part at C-4, the linker at C-5 or C-6 and the terminal hydrophilic moiety were necessary for pyrimidine derivatives to gain effective inhibitory activity against both enzymes (Fig. 1). Based on this hypothesis, we decided to utilize the 4-benzyloxy aniline moiety of Lapatinib to maximize the chances of identifying

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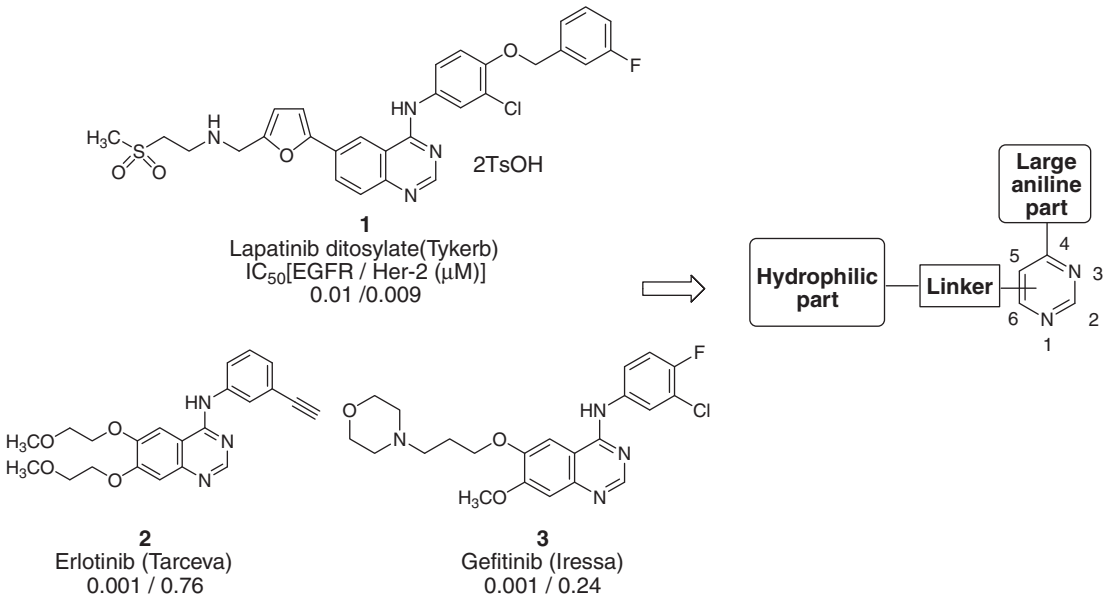


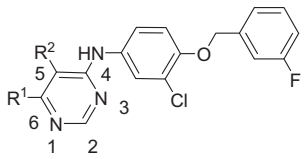
Figure 1. Examples of clinical ErbB family inhibitors and the putative pharmacophore model of the pyrimidine analogue.

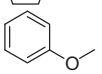
a dual inhibitor, and focus our SAR effort on the C-5 and C-6 substituents of the pyrimidine ring.

We first explored the C-6 substituent (Table 1). These compounds were evaluated for their inhibitory activity toward tyrosine phosphorylation of the EGFR and Her-2 kinases by homogeneous time-resolved fluorescence (HTRF) assay.[13] IC_{50} values were calculated using the XLFit (IDBS, Guildford, United Kingdom) curve-fitting tool from Microsoft Excel. The parent pyrimidine compound **4** exhibited modest inhibitory activity against EGFR and low potency against Her-2. Methyl or phenyl substitution at C-6 maintained the EGFR activity, and showed the modest improvement of the potency against Her-2 (**5**, **6**). Compounds containing heteroaryl, amime or phenol at the C-6 position were not potent inhibitors, especially against Her-2 (**7**–**10**). Incorporation of a 5-methyl group into compound **5** provided analogue **11** which showed significant improvement in potency against both enzymes. However, the corresponding 6-ethyl and the 6-phenyl derivatives were poorer as inhibitors of both EGFR and Her-2 (**12**, **13**). Such a pronounced drop in activity against the enzymes suggested that the C-6 position of the pyrimidine nucleus is close to the hinge region of EGFR or Her-2, and it would be difficult to introduce a linker moiety at this position. Unfortunately, compound **11** did not exhibit effective antiproliferative activity against the Her-2 dependent cell line (BT474) (data not shown). Therefore, we turned our attention to explore the C-5 substituent of the 6-methylpyrimidine analogue.

We thought that the linker in our pyrimidine series should be in similar orientation as the phenyl ring of quinazoline, that is, a substituent at the C-5 position was required to be coplanar with the pyrimidine ring. Thus, we tried to find a linker that could maintain coplanarity with the pyrimidine nucleus. Table 2 summarizes the primary SAR of different linkers at the C-5 position. We carried out this exploration using a morpholine substituent at the terminal position because it was utilized as the hydrophilic group in gefiti-

Table 1
Structure–activity relationships for the C-6 position



Compound	R^1	R^2	EGFR IC_{50}[a] (μM)	Her-2 IC_{50}[a] (μM)	Compound	R^1	R^2	EGFR IC_{50}[a] (μM)	Her-2 IC_{50}[a] (μM)
4	H	H	0.92	25	**9**		H	>100	>100
5	CH_3	H	1.1	6.5	**10**		H	10	>100
6		H	1.2	6.3	**11**	CH_3	CH_3	0.017	0.14
7		H	47	>100	**12**	CH_3CH_2	CH_3	0.64	2.9
8		H	22	36	**13**		CH_3	20	65

[a] $n = 3$.

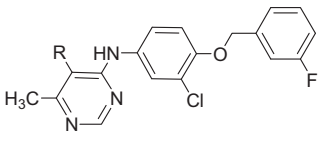
nib and could be introduced onto the terminal part easily. The aliphatic alkyne compound (**17**) and acrylamide derivative (**19**) displayed effective EGFR and Her-2 dual inhibitory activity, while the phenylacetylene (**18**) and styrene (**20**) derivatives exhibited lower inhibitory potency. Oxime analogues (**14**, **15**) which were expected to hold similar orientation with alkenyl derivatives, exhibited lower potency than **17**. Phenyl ring substitution did not showed effective inhibitory activity because of the difficulty of holding coplanarity (**16**). These SAR results indicated that an alkenyl or alkynyl pyrimidine moiety mimicked the quinazoline scaffold well, and these derivatives could bind to EGFR or Her-2 in a similar fashion to Lapatinib.

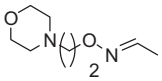
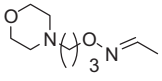
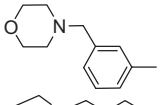
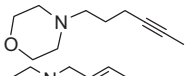
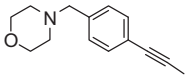
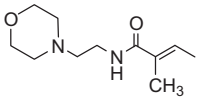
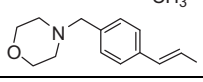
This encouraged us to further pursue SAR within acrylamide and aliphatic alkyne derivatives. In the acrylamide series (Table 3), replacement of the α-methyl with α-fluoro retained the inhibitory activities against both receptors (**21**), but the desmethyl derivative (**22**) decreased the potency. Changing the terminal morpholine in **21** to pyrrolidine resulted in an approximately sevenfold increase in inhibitory activity against both receptors (**23**), while the non-basic methylsulfone compound **24** displayed a slight increase in potency. This result suggested that the terminal amine group is not essential for the EGFR/Her-2 dual inhibitor. On the other hand, the conformationally restricted amide compound **25** exhibited reduced potency against EGFR and Her-2. Thus, the direction of the hydrophilic part may influence the inhibitory ability of these kinases.

The SAR of aliphatic alkyne derivatives is presented in Table 4. Shortening the length between the sp carbon and morpholine by one methylene maintained the inhibitory activities against both receptors (**26**), but truncating two methylenes resulted in significant loss of potency (**27**). In addition, insertion of an oxime moiety led to retention of the potency against EGFR and Her-2 (**28**). These results indicated that the length of this part needed more than two carbons. In terms of the terminal hydrophilic part, the pyrrolidine analogue (**29**) exhibited similar activity to compound **17**, while the *N*-ethylpiperadine derivative (**30**) displayed slight improvement in potency against both EGFR and Her-2. However, the benzylamine analogue (**31**) decreased the inhibitory activities against both receptors.

A proposed binding mode for compound **30**, modeled on the Lapatinib/EGFR X-ray structure (PDB code 1XKK),[14] is shown in Figure 2. In this model, the pyrimidine core and aniline portion

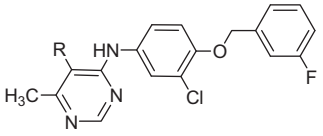
Table 2
Structure–activity relationships for the C-5 linker part

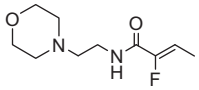
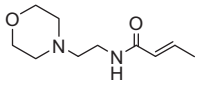
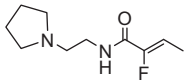
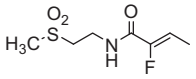
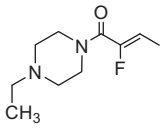


Compound	R	EGFR IC_{50}[a] (μM)	Her-2 IC_{50}[a] (μM)
14		0.068	0.74
15		0.065	1.8
16		0.59	3.75
17		0.034	0.15
18		0.24	0.44
19		0.028	0.081
20		0.12	0.19

[a] $n = 3$.

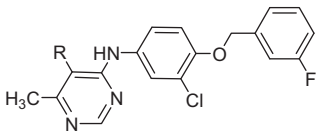
Table 3
Structure–activity relationships for the alkenyl pyrimidines

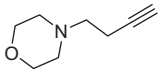
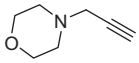
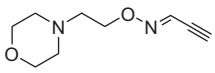
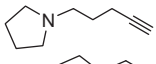
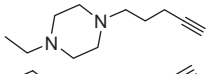
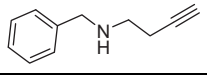


Compound	R	EGFR IC_{50}[a] (μM)	Her-2 IC_{50}[a] (μM)
21		0.018	0.078
22		0.114	0.318
23		0.003	0.019
24		0.019	0.071
25		0.156	0.102

[a] $n = 3$.

Table 4
Structure–activity relationships for the alkynyl pyrimidines



Compound	R	EGFR IC_{50}[a] (μM)	Her-2 IC_{50}[a] (μM)
26		0.013	0.074
27		4.13	5.38
28		0.038	0.070
29		0.011	0.095
30		0.009	0.036
31		0.27	0.81

[a] $n = 3$.

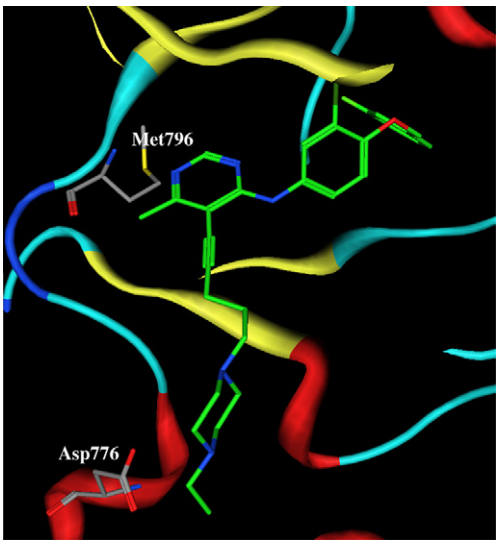


Figure 2. Alkyne analogue **30** docked into the X-ray structure of EGFR/Lapatinib.

are oriented in a manner similar to Lapatinib. The C-5 linker extends out of the ATP pocket into the solvent exposed region, and the alkynyl moiety is coplanar with the pyrimidine ring, as we predicted. The terminal pyrrolidine moiety is in the proximity of Asp 776, suggesting that the pyrrolidine can form ionic interactions with this residue. Therefore, the length of the spacer between the sp2 or sp carbon and terminal hydrophilic group may influence the enzyme inhibitory activity, and the SARs of acrylamide and aliphatic alkyne analogues agree with this proposed model.

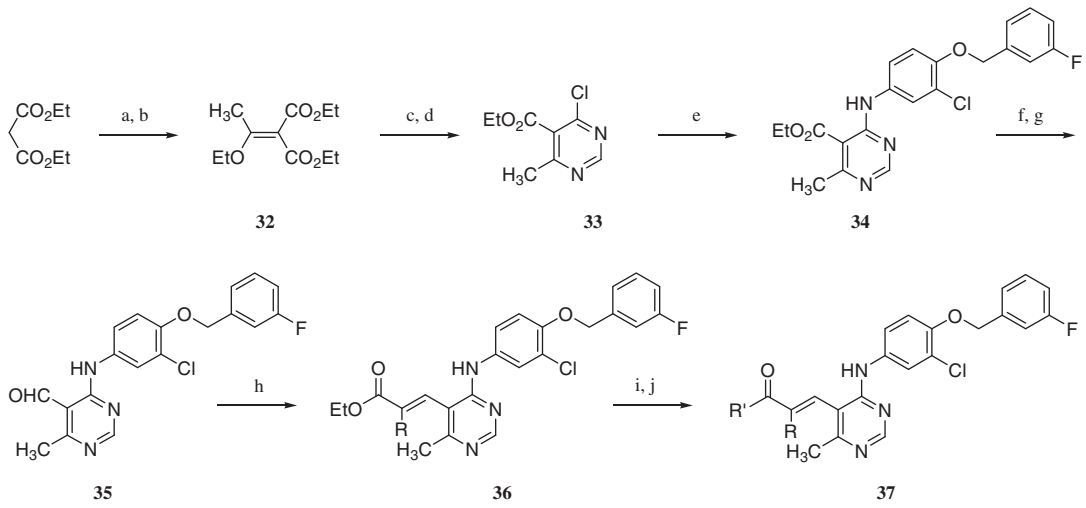
The antiproliferative activities for BT474 for selected compounds are shown in Table 5.[4] Compound **23** and **30** showed significant loss of potency compared to their enzyme inhibitory activities [BT474/Her-2: 59.4 (**23**), 40.00 (**30**)]. On the other hand, compound **18** and **20** inhibited the growth of BT474 as expected from their potencies of enzyme inhibition [BT474/Her-2: 0.900 (**18**), 3.39 (**20**)]. The reason for this is not clear, but cellular permeability might influence the antiproliferative activity. Compound **18** and **20**, containing a phenyl ring in the linker part were more lipophilic than **23** and **30** [*S* log *P*:[15] 6.96 (**18**), 7.73 (**20**) vs 5.98 (**23**), 6.23 (**30**)], and might have an advantage in penetrating the cell membrane. In addition, compound **18** had a good aqueous solubility compared to Lapatinib. Therefore, we thought that the further exploration of the 5-alkenyl pyrimidine analogue had promise for the discovery of well-balanced compound in terms of the antiproliferative potency and physicochemical properties.

The synthetic route to the 5-acrylamide analogues is shown Scheme 1. Compound **32** was prepared from commercially available diethyl malonate by a two-step reaction sequence. Ring closure reaction with formamidine followed by the chlorination with $POCl_3$ provided 4-chloropyrimidine (**33**). This intermediate was coupled with the 4-benzyloxy aniline[16] to afford compound

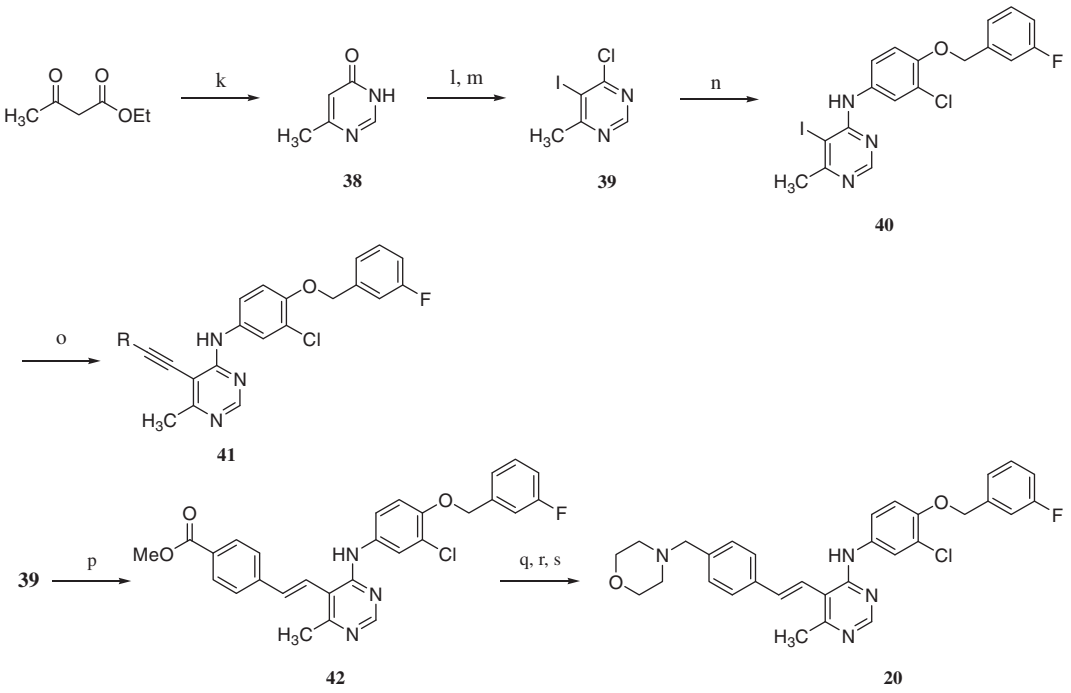
Table 5
Inhibition of BT474 proliferation by dual inhibitors

Compound	EGFR IC_{50} (μM)	Her-2 IC_{50} (μM)	BT474 IC_{50}[a] (μM)	Ratio BT474/Her-2	*S* log *P*	Solubility[b] (μM)
18	0.24	0.44	0.394	0.900	6.96	>50
20	0.12	0.19	0.644	3.39	7.73	2[d]
23	0.003	0.019	1.13	59.4	5.98	>50
30	0.009	0.036	1.44	40.0	6.23	>50
Lapatinib[c]	0.010	0.009	0.10	11.11	6.67	24[e]

[a] $n = 1$.
[b] The solubility is measured in high-speed solubility screening assay using DMSO solutions. The solvent is Japanese Pharmacopoeia XIV (JP) disintegration media JP 2nd-fluid (JP2; pH 6.8) plus Taurocholic acid.
[c] Ref. 4.
[d] Solubility of HCl salt.
[e] In-house data.



Scheme 1. Reagents and conditions: (a) acetyl chloride (1 equiv), Et_3N (2 equiv), $MgCl_2$ (1 equiv), MeCN, 0 °C, 100%; (b) Et_2SO_4 (1.2 equiv), K_2CO_3 (1.2 equiv), DMF, 60 °C, 66%; (c) formamidine acetate (1.2 equiv), K^{t}OBu (2.2 equiv), EtOH, rt, 73%; (d) $POCl_3$ (2 equiv), toluene, 120 °C, 43%; (e) $ArNH_2$ (1 equiv), DIPEA (1 equiv), toluene, reflux, 67%; (f) $LiAlH_4$ (1.5 equiv), THF, rt, 94%; (g) $(COCl)_2$ (1.5 equiv), DMSO (3 equiv), Et_3N (5 equiv), CH_2Cl_2, −78 °C to rt, 55%; (h) phosphonate (1.1 equiv), NaH (1.1 equiv), DMF, rt, 85–95%; (i) 2 N NaOH (2 equiv), THF, MeOH, 60 °C, 100%; (j) amine (1 equiv), HOBt (1 equiv), WSCD·HCl (1 equiv), Et_3N (2 equiv), CH_2Cl_2, rt, 40–90%.



Scheme 2. Reagents and conditions: (k) formamidine acetate (1.2 equiv), Na (2.2 equiv), EtOH, rt, 75%; (l) ICl (1 equiv), AcOH, 60 °C, 100%; (m) $POCl_3$ (2 equiv), toluene, rt, 35%; (n) $ArNH_2$ (1 equiv), EtOH, reflux, 54%; (o) alkyne (1.5 equiv), $PdCl_2(PPh_3)_2$ (5 mol %), CuI (10 mol %), Et_3N (5 equiv), DMF, 80 °C, 50–90%; (p) phenylvinylbronic acid pinacol ester (1.2 equiv), $Pd(OAc)_2$ (10 mol %), PPh_3 (30 mol %), 2 M K_2CO_3 (4 equiv), DMF, 100 °C, 65%; (q) $LiAlH_4$ (1.5 equiv), THF, rt, 77%; (r) MnO_2 (10 equiv), THF, rt, 77%; (s) morpholine (4 equiv), $NaBH(OAc)_3$ (4 equiv), AcOH (1 equiv), rt to 50 °C, 82%.

34. Reduction of **34** with $LiAlH_4$, followed by Swern oxidation provided the aldehyde **35**. Horner–Wadsworth–Emmons reaction of **35** with phosphonates afforded the acrylate **36**. Hydrolysis of **36** with NaOH followed by condensation with the amines provided the acrylamide analogues (**37**).

The synthetic route to 5-alkynyl analogues and compound **20** is shown Scheme 2. 6-Methyl pyrimidone (**38**) was obtained by the reaction of ethyl acetoacetate with formamidine acetate under basic condition. Treatment of **38** with ICl was followed by chlorination with $POCl_3$ to give 5-iodo-4-chloro-6-methylpyrimidine (**39**). The reaction of **39** with the 4-benzyloxy aniline provided **40**. Sonogashira coupling reaction of **40** with alkynes provided 5-alkynyl analogues (**41**). On the other hand, Suzuki–Miyaura coupling reaction of **39** with phenylvinylborate provided compound **42**. Conversion of the terminal methyl ester into aldehyde, followed by reductive amination with morpholine provided compound **20**.

In summary, we have described early SARs leading to novel alkenyl and alkynyl pyrimidine compounds which have effective EGFR/Her-2 dual inhibitory activity. The key modification was the introduction of a 5-alkenyl or alkynyl moiety that contained hydrophilic groups at the appropriate position. Although acrylamide and aliphatic alkyne analogues did not exhibit potent antiproliferative activity against BT474, we found that introducing an aromatic ring into the terminal hydrophilic part could improve cellular activity. Further exploration of these 4-anilinopyrimidine derivatives as EGFR/Her-2 dual inhibitors will be reported in due course.

References and notes

1. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Patents* **2004**, *14*, 35.
2. Olayioye, M. A.; Neve, R. M.; Lane, H. A.; Hynes, N. E. *EMBO J.* **2000**, *19*, 3159.
3. Kersemaekers, A.-M. F.; Fleuren, G. J.; Kenter, G. G.; Van den Broek, L. J. C. M.; Uljee, S. M.; Hermans, J.; Van de Vijver, M. J. *Clin. Cancer Res.* **1999**, *5*, 577.
4. Rusnak, D. W.; Lackey, K.; Affleck, K.; Wood, E. R.; Alligood, K. J.; Rhodes, N.; Keith, B. R.; Murray, D. M.; Glennon, K.; Knight, W. B.; Mullin, R. J.; Gilmer, T. M. *Mol. Cancer Ther.* **2001**, *1*, 85.
5. Fabian, M. A.; Biggs, W. H., III; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. *Nat. Biotechnol.* **2005**, *23*, 329.
6. Liao, J. J. *J. Med. Chem.* **2007**, *50*, 409.
7. (a) Bergman, E.; Forsell, P.; Persson, E. M.; Knutson, L.; Dickinson, P.; Smith, R.; Swaisland, H.; Farmer, M. R.; Cantarini, M. V.; Lennernäs, H. *Int. J. Pharm.* **2007**, *341*, 134; (b) Koch, K. M.; Reddy, N. J.; Cohen, R. B.; Lewis, N. L.; Whitehead, B.; Mackay, K.; Stead, A.; Beelen, A. P.; Lewis, L. D. *J. Clin. Oncol.* **2009**, *27*, 1191; (c) Bubendorf, A. G.; Hennig, M.; Hidber, P.; Rimmler, G.; Rohrer, F. WO 2004072049 A1.
8. (a) Gavai, A. V.; Fink, B. E.; Fairfax, D. J.; Martin, G. S.; Rossiter, L. M.; Holst, C. L.; Kim, S.-H.; Leavitt, K. J.; Mastalerz, H.; Han, W.-C.; Norris, D.; Goyal, B.; Swaminathan, S.; Patel, B.; Mathur, A.; Vyas, D. M.; Tokarski, J. S.; Yu, C.; Oppenheimer, S.; Zhang, H.; Marathe, P.; Fargnoli, J.; Lee, F. Y.; Wong, T. W.; Vite, G. D. *J. Med. Chem.* **2009**, *52*, 6527; (b) Rheault, T. R.; Caferro, T. R.; Dickerson, S. H.; Donaldson, K. H.; Gaul, M. D.; Goetz, A. S.; Mullin, R. J.; McDonald, O. B.; Petrov, K. G.; Rusnak, D. W.; Shewchuk, L. M.; Spehar, G. M.; Truesdale, A. T.; Vanderwall, D. E.; Wood, E. R.; Uehling, D. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 817; (c) Lin, R.; Johnson, S. G.; Connolly, P. J.; Wetter, S. K.; Binnun, E.; Hughes, T. V.; Murray, W. V.; Pandey, N. B.; Moreno-Mazza, S. J.; Adams, M.; Fuentes-Pasquera, S. A.; Middleston, S. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2333.
9. (a) Waterson, A. G.; Stevens, K. L.; Reno, M. J.; Zhang, Y.-M.; Boros, E. E.; Frederic, B.; Rastagar, A.; Uehling, D. E.; Dickerson, S. H.; Reep, B.; McDonald, O. B.; Wood, E. R.; Rusnak, D. W.; Alligood, K. J.; Rudolph, S. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2419; (b) Xu, G.; Searle, L. L.; Hughes, T. V.; Beck, A. K.; Connolly, P. J.; Abad, M. C.; Neepe, M. P.; Struble, G. T.; Springer, B. A.; Emanuel, S. L.; Gruninger, R. H.; Pandey, N.; Adams, M.; Moreno-Mazza, S.; Fuentes-Pesquera, A. R.; Middleton, S. A.; Greenberger, L. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3495; (c) Xu, G.; Abad, M. C.; Connolly, P. J.; Neeper, M. P.; Struble, G. T.; Springer, B. A.; Emanuel, S. L.; Pandey, N.; Gruninger, R. H.; Adams, M.; Moreno-Mazza, S.; Fuentes-Pesquera, A. R.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4615; (d) Hughes, T. V.; Xu, G.; Wetter, S. K.; Connolly, P. J.; Emanuel, S. L.; Karnachi, P.; Pollack, S. R.; Pandey, N.; Adams, M.; Moreno-Mazza, S.; Middleton, S. A.; Greenberger, L. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4896.
10. Wakeling, A. E.; Guy, S. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Barker, A. J.; Gibson, K. H. *Cancer Res.* **2002**, *62*, 5749.
11. Moyer, J. D.; Barbacci, E. G.; Iwata, K. K.; Arnold, L.; Boman, B.; Cunningham, A.; DiOrio, C.; Doty, J.; Morin, M. J.; Moyer, M. P.; Neveu, M.; Pollack, V. A.; Pustilnik, L. R.; Reynolds, M. M.; Slaon, D.; Theleman, A.; Miller, P. *Cancer Res.* **1997**, *57*, 4838.
12. (a) Zhang, Y.-M.; Cockerill, S.; Guntrip, S. B.; Rusnak, D.; Smith, K.; Vanderwall, D.; Wood, E.; Lackey, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 111; (b) Rusnak, D. W.; Affleck, K.; Cockerill, S. G.; Stubberfield, C.; Harris, R.; Page, M.; Smith, K. J.;

Guntrip, S. B.; Carter, M. C.; Shaw, R. J.; Jowett, A.; Stables, J.; Topley, P.; Wood, E. R.; Brignola, P. S.; Kadwell, S. H.; Reep, B. R.; Mullin, R. J.; Alligood, K. J.; Keith, B. R.; Crosby, R. M.; Murray, D. M.; Knight, W. B.; Gilmer, T. M.; Lackey, K. *Cancer Res.* **2001**, *61*, 7196.
13. Kolb, A. J.; Kaplita, P. V.; Hayes, D. J.; Park, Y.-W.; Pernell, C.; Major, J. S.; Mathis, G. *Drug Discovery Today* **1998**, *7*, 333.
14. Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. *Cancer Res.* **2004**, *64*, 6652–6659.
15. Wildman, S. A.; Crippen, G. M. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868.
16. Carter, M. C.; Cockerill, G. S.; Guntrip, S. B.; Lackey, K. E.; Smith, K. J. WO 9935146 A1.