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New dual inhibitors of EGFR and HER2 protein tyrosine kinases

Brian E. Fink,* Gregory D. Vite, Harold Mastalerz, John F. Kadow, Soong-Hoon Kim, Kenneth J. Leavitt, Karen Du, Donald Crews, Toomas Mitt, Tai W. Wong, John T. Hunt, Dolatrai M. Vyas and John S. Tokarski

Departments of Oncology Chemistry, Discovery Biology, and Computer Aided Drug Design, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543-4000, USA

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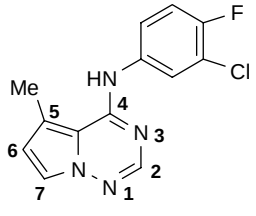
Abstract—A novel series of dual EGFR and HER2 inhibitors based on the pyrrolo[2,1-*f*][1,2,4]triazine nucleus is described. A general route toward their synthesis, which enables functionalization at multiple sites, has been developed. Biological evaluation in enzymatic and cell-based assays has identified a series of C-6 carbamates with potent biochemical and cellular activities.

The role of receptor tyrosine kinases (RTK) as key regulators of the cellular processes governing proliferation and differentiation has led to intensive efforts focused on identifying selective inhibitors for use in cancer treatments.[1] Among the known RTKs, the ErbB protein kinases are one of the most studied cell signaling families. The ErbB family consists of four receptors: epidermal growth factor receptor (EGFR/ErbB-1), HER2 (ErbB-2/neu), ErbB-3, and ErbB-4. There is considerable evidence, both preclinical and clinical, associating EGFR and HER2 with the occurrence and progression of certain cancers.[2] Current therapies using either monoclonal antibodies (cetuximab and trastuzumab) or small molecules (erlotinib) to selectively target EGFR or HER2 have demonstrated success in the clinic.[3] However, both EGFR and HER2 may act in a synergistic manner through the formation of heterodimers to produce mitogenic signaling in excess of that produced from either receptor alone.[4] Additionally, overexpression of either or both EGFR and HER2 is often correlated with poor response to chemotherapeutic agents and overall poor patient prognosis.[5] Targeting both EGFR and HER2 has therefore become a promising approach to cancer treatment.

Recently, we identified the pyrrolo[2,1-*f*][1,2,4]triazine nucleus as a novel scaffold for ATP-competitive kinase inhibitors.[6] We demonstrated that variation of the C-4 aniline resulted in compounds with potent and selective biochemical inhibition of VEGFR-2 or EGFR (Fig. 1). In addition, earlier SAR indicated that the C-5 and C-6 positions were tolerant of substitution and might be promising sites for introduction of functionality to modulate the physicochemical properties of the core. Here, we report on our efforts to extend further the use of the pyrrolotriazine core to the development of a compound with dual EGFR and HER2 activities.

The goal of the present work was to improve on the EGFR biochemical activity originally found in **1**, while incorporating HER2 activity and improving the overall physicochemical properties of the molecule. A proposed binding mode for **1**, modeled after the erlotinib/EGFR X-ray structure,[7] is shown in Figure 2.



1
EGFR IC_{50}: 0.100 μM
VEGFR-2 IC_{50}: >10 μM

Figure 1. Novel kinase inhibitor template.

Keywords: panHER; EGFR; Kinase inhibitor.
* Corresponding author. Tel.: +1 6092523420; fax: +1 6092526601; e-mail: brian.fink@bms.com

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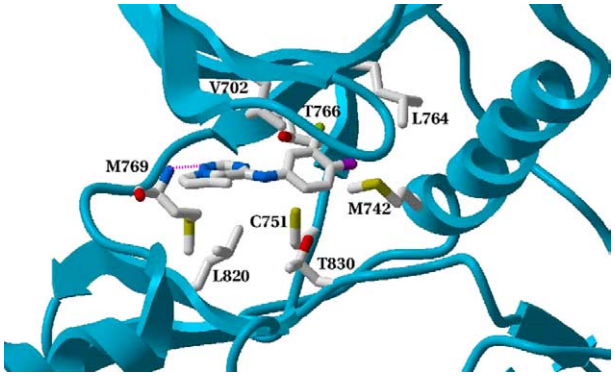


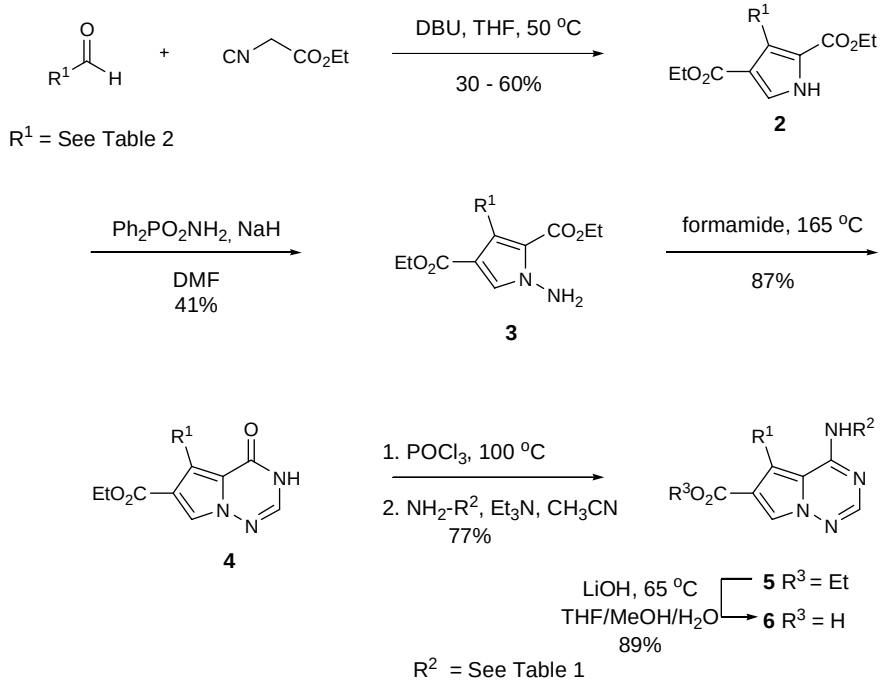
Figure 2. Predicted binding mode of compound **1** in the X-ray structure of HER1 based on co-crystal structures of similar kinase inhibitors. A few select residues are shown for orientation.[7]

In this model, the pyrrolotriazine core is oriented in the ATP-binding site such that N-1 makes a hydrogen bond with the hinge region M769 carbonyl in a manner similar to the N-1 of the quinazoline core. The aniline portion of the molecule is then oriented into a hydrophobic pocket deep within the binding site. Presumably the size and functionality of this pocket are important contributors to the selectivity of an agent for a specific kinase. Substitution at the C-5 position is directed into a smaller hydrophobic pocket flanked by V702 and L820, and is roughly equivalent to the ribose binding region of ATP. In this binding mode, the C-6 position points toward surface-exposed protein and was deemed to be an appropriate position to incorporate polar/solubilizing functionality.

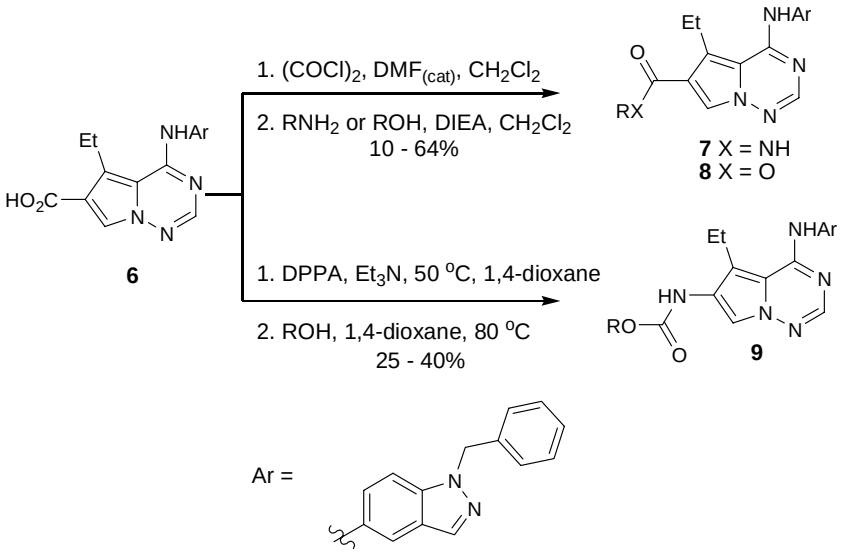
To simultaneously functionalize the 4-, 5-, and 6-positions, a general synthetic method was developed that would allow for incorporation of additional functionality at these positions (Scheme 1). The reaction of two equivalents of ethylisocyanoacetate with a variety of aldehydes in the presence of DBU afforded, in a single step, moderate to good yield of pyrrolodiesters **2**.[8] Deprotonation of **2** and N-amination with *O*-(diphenylphosphinyl)-hydroxylamine[9] afforded a series of *N*-aminopyrroles **3**, that were readily cyclized in formamide to afford the pyrrolotriazinone cores **4**.

Activation of the 4-position of intermediate **4** via the corresponding chloroimidate using neat $POCl_3$ was followed by addition of an amine in acetonitrile to afford pyrrolotriazines **5**. Various functional groups were investigated at the 6-position to determine their effect on activity and their viability as linkers to append additional polar/solubilizing groups. Hydrolysis of **5** with LiOH afforded the carboxylic acid **6**, which was coupled to amines or alcohols via the corresponding acid chloride to afford a series of amides **7** or esters **8** (Scheme 2). Alternatively, Curtius rearrangement of **6** with DPPA and reaction of the intermediate isocyanate with an alcohol afforded a series of carbamates **9**. Reduction of **5e** with DIBAL-H to alcohol **10**, followed by MnO_2 oxidation and reductive amination, afforded amine **11** (Scheme 3). Alkylation of **10** with ethyl iodide afforded an ether analog **12**. Activation of **10** with neat $SOCl_2$, followed by treatment with NaCN and acidic hydrolysis, afforded a separable mixture of **13** and **14**, corresponding to one carbon homologated analogs of **6** and **7**.

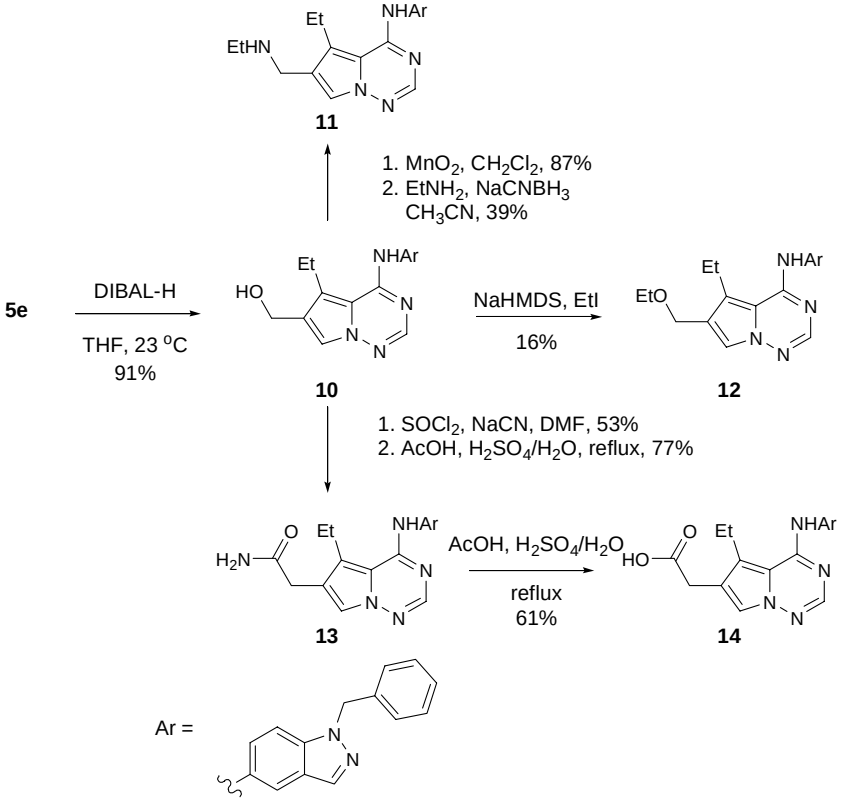
In pursuit of our goal to improve both EGFR and HER2 activities in this series, biochemical activities were



Scheme 1.



Scheme 2.

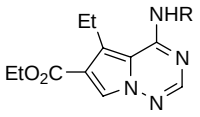


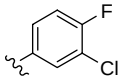
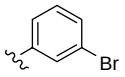
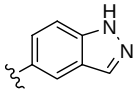
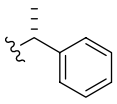
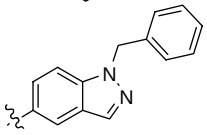
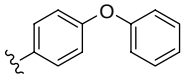
Scheme 3.

assessed using in vitro enzyme assay methods previously reported.[6,10]

Based on our proposed binding mode, substitution at the C-4 position was seen as an area likely to influence both the potency and selectivity of this series (Table 1). The SAR at position 4, relative to EGFR, indicates that enzyme activity is effectively inhibited when the C-4 substituent is a relatively small aniline or fused 5,6-bicyclic system (**5a**, **5b** or **5c**). However, this activity was somewhat attenuated if the aromatic ring was displaced by one carbon (**5d**). Additional lipophilic aryl groups were tolerated (**5e**), but the nature and orientation of the added aryl group appear to be important for potency (**5f**). The SAR, relative to HER2, is somewhat more straightforward. As the size increased, inhibitory potency versus HER2 followed in parallel. While **5a**–**5d** showed modest activity against HER2, increasing

Table 1. Structure–activity relationship for the C-4 position



Compound	R	HER2[a,b] IC_{50} (μM)	EGFR[b] IC_{50} (μM)
5a		2.8	0.09
5b		3.0	0.06
5c		3.3	0.05
5d		7.2	0.20
5e		0.18	0.12
5f		0.82	0.61

[a] Recombinant HER2 cytoplasmic sequence is expressed in Sf9 insect cells as an untagged protein and purified by ion-exchange chromatography. HER2 kinase activity is measured under the same conditions as for EGFR. See Refs. 6 and 10 for assay conditions.

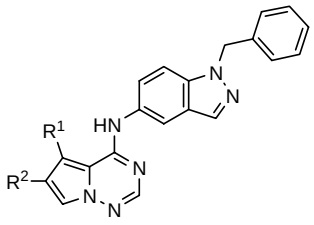
[b] IC_{50} values are reported as the mean of at least three determinations. Variability around the mean value was <15%.

steric bulk and overall lipophilicity, as with *N*-benzyl indazole **5e** or biaryl ether **5f**, greatly improved activity. A similar trend was recently reported in a quinazoline series where increasing size at the C-4 aniline position substantially reduced EGFR activity and moderately improved HER2 activity.[11]

The origins of this effect are unclear, given the sequence similarities between EGFR and HER2 in this region, the only difference being Cys775 (EGFR) versus Ser783 (HER2). It has been suggested that these residues, along with two threonine residues, form a water-mediated hydrogen bonding network that contributes to the shape and polarity of the hydrophobic pocket.[11] Perhaps interaction with this network, in the Ser-containing HER2, requires additional binding affinity that can only be attained through incorporation of a second distal ring in HER2. Given the overall plasticity of receptor tyrosine kinases, sequence differences in regions distal to the ATP-binding site may influence the size of this binding pocket. Further explanation may have to await more complete HER2 structural information.

Since good biochemical potency was achieved against both receptors when the *N*-benzylindazole was used at the C-4 position, further SAR was developed within that series. A survey of substitution patterns at the C-5 positions showed that both receptors tolerate a variety of

Table 2. Structure–activity relationship for the C-5 and C-6 positions



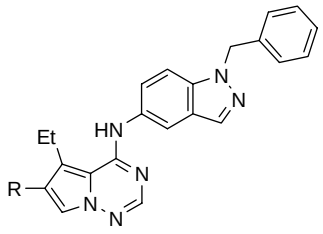
Compound	R^1	R^2	HER2 IC_{50}[a] (μM)	EGFR IC_{50}[a] (μM)
5e	Et	CO_2Et	0.20	0.20
5g	H	CO_2Et	0.39	0.12
5h	Me	CO_2Et	0.20	0.20
5i	*i*Pr	CO_2Et	0.21	0.20
5j	*n*Pr	CO_2Et	1.2	0.95
5k	Bn	CO_2Et	>25	>25
6	Et	CO_2H	0.04	0.02
7a	Et	$C(O)NH_2$	0.11	0.12
7b	Et	C(O)NHMe	0.13	0.07
7c	Et	$C(O)NMe_2$	0.50	0.20
9a	Et	NHC(O)OBn	0.17	0.21
10	Et	CH_2OH	0.73	0.49
11	Et	CH_2NHEt	9.7	7.4
12	Et	CH_2OEt	1.4	0.95
13	Et	$CH_2C(O)NH_2$	2.4	1.1
14	Et	CH_2CO_2H	0.68	1.6

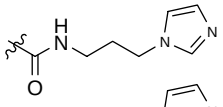
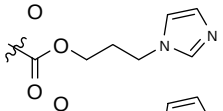
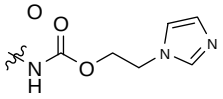
[a] IC_{50} values are reported as the mean of at least three determinations. Variability around the mean value was <15%.

small alkyl groups, such as methyl, ethyl, and isopropyl (Table 2).

Both HER2 and EGFR activity eroded with increasing size/shape of the C-5 substituent, indicating a shape preference of this pocket (compare **5i** vs. **5j**). While substitution at the C-4 and C-5 positions resulted in gains in potency, overall they did not lead to the levels of activity necessary for good cellular potency (data not shown). Since our binding model indicated that the C-6 position is directed toward solvent-exposed regions of the binding pocket, substantial gains in biochemical potency were not expected. To our surprise, carboxylic acid **6** showed a ~10-fold improvement in activity against each receptor. This result indicates that the nature of the linker group at C-6 dramatically impacts binding. Primary and secondary amides **7a** or **7b** showed similar inhibitory activity compared to the corresponding esters; however, the dimethylamide **7c** resulted in a loss of activity within this series compared to **7a** or **7b**. Interestingly, the benzyl carbamate **9a** showed activity similar to both esters and amides. Substituents lacking the ester or amide carbonyl group, for example, the ethyl ether **12** or ethylamine **11**, provided significantly reduced potency as did one atom homologation in the amide **13** or carboxylic acid **14**. These results taken together indicate a distinct requirement for the immediate C-6 linker to be planar or nearly planar (i.e., first atom attached at C-6 is preferentially sp2 hybridized) with the pyrrolotriazine ring and to contain a hydrogen bond acceptor group. These findings are reinforced by our modeling studies, which suggest that the linker atoms at the C-6 position occupy a constricted space between the N-terminal

Table 3. Effect of linker on enzymatic and cellular activity[12]



Compound	R	IC_{50}[a] (μM)			
		HER2	EGFR	BT474[b]	Sal2[c]
7d		0.03	0.04	7.0	5.8
8a		0.24	0.17	3.1	1.6
9b		0.04	0.04	0.86	0.46

[a] IC_{50} values are reported as the mean of at least three determinations. Variability around the mean value was <15%.
[b] Cell line BT474 (breast carcinoma) overexpresses HER2.[13]
[c] Cell line Sal2 (salivary gland carcinoma) contains a constitutively active HER2.[14]

and C-terminal lobes. While this position is directed toward the solvent, substituents can therefore still interact with the protein.

Since simple amides, esters, and carbamates showed equivalent activity, several small libraries were prepared using these groups to append polar/solubilizing groups to the core. Table 3 shows a representative series of homologous imidazole-substituted compounds containing each of the three linkers. While the imidazole group had modest effects when linked through an ester (**8a**), improved biochemical potency was observed in both the amide **7d** and the carbamate **9b** cases. In addition, **9b** showed good anti-proliferative activity in both the HER2-driven BT474 (breast carcinoma) and Sal2 (salivary gland carcinoma) cell lines,[14] with 10-fold better potency over the analogous amide **7d**. Given the similarity of the two compounds, in terms of structure, physical properties, and biochemical activity, the reason for the improved cellular activity is unclear. Western blot analysis of phosphorylated HER2 protein in BT474 cells showed significantly lower levels of phosphorylation upon treatment with **9b**, relative to **7d** (data not shown). Therefore, the improvement in cellular activity for **9b** may simply reflect improved cell penetration.

Compound **9b** was screened against a small panel of tyrosine and serine/threonine kinases. As shown in Table 4, **9b** showed >200-fold selectivity for EGFR and HER2.

The pyrrolo[2,1-*f*][1,2,4]triazine nucleus has proven to be a versatile scaffold for the development of potent tyrosine kinase inhibitors. We have demonstrated that potent and selective dual EGFR/HER2 inhibitors may be prepared through modulation of the C-4 position, while additional potency, physical properties, and cellular activity may be modulated through the C-6 position. Use of a carbamate linker at the C-6 position was found to be optimal for appending polar functionality, a combination of which contributed favorably to the biochemical activity, as well as cellular activity in the BT474 and Sal2 cell lines.

Table 4. Kinase selectivity profile for compound **9b**

Kinase	IC_{50}[a] (μM)
EGFR	0.04
HER2	0.04
Met	>10
FAK	>25
p38	>25
MAPKAP kinase 2	>25
IGF-1R	>25

[a] IC_{50} values are reported as the mean of at least three determinations. Variability around the mean value was <15%.

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