Bioorganic & Medicinal Chemistry Letters 18 (2008) 4896–4899

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A novel 5-[1,3,4-oxadiazol-2-yl]-*N*-aryl-4,6-pyrimidine diamine having dual EGFR/HER2 kinase activity: Design, synthesis, and biological activity

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ARTICLE INFO

Article history:
Received 28 April 2008
Revised 10 July 2008
Accepted 14 July 2008
Available online 17 July 2008

Keywords:
EGFR
HER2
HER1
Kinase
Pyrimidine
Quinazoline mimic
Intramolecular hydrogen bond
Restricted rotation
Oxadiazole

ABSTRACT

A novel 5-[1,3,4-oxadiazol-2-yl]-*N*-aryl-4,6-pyrimidine diamine was synthesized and found to have potent dual EGFR/HER2 kinase inhibitory activity. The structure-based drug design of this molecule as well as the kinase and cellular inhibition of HER2 kinase dependent cell lines will be discussed.

There is a need to identify safe and effective compounds to reduce the abnormal cell proliferation that is characteristic of cancer by targeting the genetic basis for the disease. Small molecule kinase inhibitors have potential to treat specific genetic alterations that play a role in the pathogenesis of human malignancies, and may provide effective, low-toxicity therapies to inhibit tumor growth. Inhibition of protein kinase activity has been a successful approach for treatment of cancer and inflammatory disease.[1] HER1 and HER2 are members of the epidermal growth factor receptor family that are involved in the development and progression of several human cancers including lung and breast.[2] These receptors are regulated by kinase activity that can be inhibited by small molecule inhibitors. To that end, we concentrated our efforts to discover novel inhibitors of the HER kinases. The HER receptors can be activated through homo- or heterodimerization with other HER receptors resulting in phosphorylation events and downstream signaling that produces excessive growth by inducing cell proliferation and inhibiting apoptotic pathways. The HER family of kinases can stimulate growth through heterodimerization with other HER family members even when one of the receptors, for example, HER1 (EGFR), has been inhibited by a small molecule (or monoclonal antibody). Therefore, we focused on finding a small molecule HER kinase inhibitor with activity against all HER family members to prevent transactivation across all receptor isoforms. Specifically, we screened our compound library for the inhibition of EGFR and HER2 as they are hyper-activated in several cancers, and their over-expression is frequently associated with poor prognosis.[3,4]

An intermediate in a related oncology program, compound **1** (Fig. 1), was identified as a hit for HER family kinase inhibition with

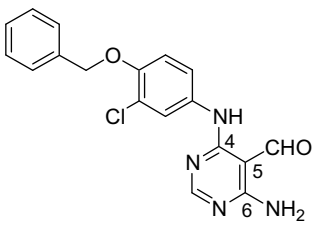

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Figure 1. Screening hit compound **1**.

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0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.07.057

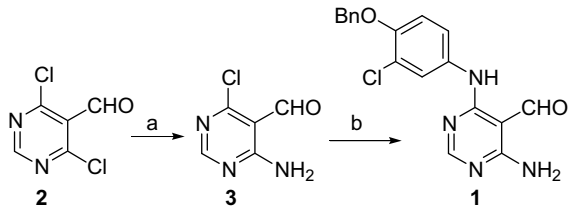
an IC_{50} value of 0.076 μM against EGFR and modest HER2 activity (63% inhibition at 100 μM).[5] With **1** in hand we modified the substituent at the 5-position of the pyrimidine ring in an effort to improve HER2 kinase activity in the series.

The synthesis of **1** is outlined in Scheme 1. The commercially available dichloropyrimidine **2** was treated with ammonia gas and warmed in toluene at 60 °C, leading to the selective displacement of only one of the chlorine atoms.[6] The product, 4-amino-6-chloro-pyrimidine-5-carbaldehyde (**3**), was reacted with commercially available 4-benzyloxy-3-chloro-phenylamine in DMSO at 100 °C in the presence of Et_3N to give pyrimidine **1**.

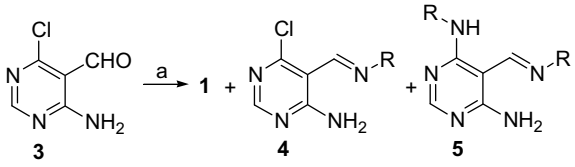
The conversion of **3** to **1** was highly dependent in the order of addition for the reagents (Scheme 2). The conversion of **3** to **1** occurred smoothly if the aniline is added last; however, if the Et_3N was added last or omitted **4** was a significant product of the reaction (Table 1). Presumably, the small amount of HCl that was formed from the desired conversion of **3** to **1** catalyzed the formation of the imine **4** as well as the formation of **5**, which is the imine of **1**, unless the acid was neutralized by the addition of a base. Remarkably, the formation of the imine **4** that occurred readily at 25 °C, in the absence of base, was not a significant product of the reaction at 100 °C when Et_3N was used as an acid scavenger.[7]

Initially the aldehyde functional group of **1** was used as a handle to further explore the SAR of the series. We thought that conversion of the aldehyde to a carboxylic acid derivative such as an ester or an amide would be an efficient way to generate analogues. Our synthesis of the corresponding esters and amides of analogue **1** is shown in Scheme 3.

The aldehyde **1** was converted to the methyl ester **6** by treatment with sodium cyanide and MnO_2 in MeOH/THF with one equivalent of AcOH.[8] The ester was readily hydrolyzed to carboxylic acid **7** by treatment with LiOH in MeOH/THF/H_2O. Initial attempts at amide formation using EDCI were low yielding, with



Scheme 1. Reagents and conditions: (a) NH_3 (gas), toluene, 60 °C, 87%; (b) DMSO, Et_3N, 100 °C, 4-benzyloxy-3-chloro-phenylamine, 61%.

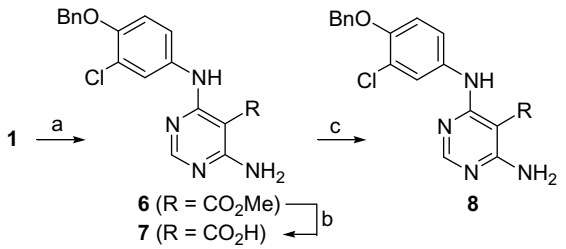


Scheme 2. Reagent and condition: (a) DMSO, No Et_3N, 25 °C, R = 4-benzyloxy-3-chloro-phenyl.

Table 1
Relative ratio[a] of products **1**, **4**, and **5** without Et_3N at 25 °C

Compound	6 min (%)	60 min (%)
1	12	10
4	39	46
5	2	20

[a] The relative ratios of products were determined by HPLC analysis.



Scheme 3. Reagents and conditions: (a) NaCN, MnO_2, MeOH, AcOH, reflux, 80%; (b) LiOH, THF, MeOH, H_2O, 25 °C, 81%; (c) HATU, EtN(*i*-Pr)$_2$, THF, $R^1NH(H)$ or R^1OH, 25 °C, 60–80%.

only small amounts of amide **8** (R = $CONHR^1$ or $CONR^1R^2$) observed. However, utilization of HATU as the coupling reagent and diisopropylethylamine in THF efficiently coupled the carboxylic acid **7** with a series of primary and secondary amines to afford various amides **8** (R = $CONHR^1$ or $CONR^1R^2$). Additionally, HATU was used to couple **7** with various alcohols to afford the corresponding esters **8** (R = CO_2R^1). The EFGR and HER2 kinase activity for a representative number of analogues of **8** is shown in Table 2. Activity against Aurora-A, CDK1, and VEGF-R2 kinases is also shown for comparison.

Although compound **1** (R = CHO) displayed good inhibition of EFGR (IC_{50} = 0.076 μM), it was only modestly potent against HER2 kinase with an IC_{50} = 1.00 μM. Therefore, our objective was to keep the EGFR potency and try to gain potency against HER2. However, none of the amides synthesized (**8b**–**8g** and **8i**) displayed inhibition of EGFR or HER2. The carboxylic acid **7** exhibited excellent potency against EGFR (IC_{50} = 41 nM), and showed a slight improvement in potency against HER2 (IC_{50} = 776 nM) compared to the lead **1**. The nitrile **8j** displayed similar activity to the carboxylic acid **7**.[9] The methyl ester **6** still retained potent EGFR activity (IC_{50} = 54 nM), and showed a marked improvement in HER2 potency (IC_{50} = 100 nM). However, the bulkier ester **8a**, which contained a 2-(morpholin-4-yl)ethylamino group, showed a loss in potency against both EFGR and HER2 (IC_{50} = 150 nM and 1.53 μM, respectively). Both the amide and ester analogues contain two heteroatoms capable of participating in H-bonds. The inactive amide analogues contain H-bond accepting O and a H-bond donating NH moieties. However, unlike the amide analogues of **8**, both heteroatoms of the ester analogues are capable of being H-bond acceptors. This difference may determine the ability of the substituent at the 5-position of **8** to be held in the same plane as the pyrimidine ring via intramolecular H-bonds. It appears that the ability of pyrimidine ring of **8** to be relatively coplanar with the substituent in the 5-position parallels the HER2 kinase activity observed. None of the analogues showed appreciable potency against the non-HER kinases Aurora-A, CDK1, or VEGF-R2.

In an effort to expand upon the improved activity of the ester analogues (Table 2) and to avoid ester related metabolism we decided to explore isosteres of esters. The oxadiazole ring has been reported as an isostere for the ester functional group.[10] Specifically, we thought that the 1,3,4-oxadiazole **11** would be an interesting analogue to pursue. Similar to an ester, compound **11** could also form two pseudocycles via intramolecular hydrogen bonds but **11** would be metabolically stable to hydrolysis.

Compound **11** was docked into a homology model of EGFR using the software GLIDE.[11–13] Molecular modeling of **11** shows two intramolecular hydrogen bonds that hold the molecule in a relatively flat pose in the ATP-site (Fig. 2).[14] Compound **11** can exist as two rotamers that are each capable of forming two intramolecular H-bonds to the oxadiazole ring. While both rotamers are probably present in solution, or in the binding site of EGFR, the more stable rotamer (~5 kcal/mol) is illustrated in Figure 2.[15] The first intramolecular hydrogen bond is between the O of the oxadiazole

Table 2
Kinase activity for analogues from Scheme 2

Compound	R	EGFR % inh. 2 μM	EGFR IC_{50}[a] (μM)	HER2 IC_{50}[a] (μM)	Aurora-A IC_{50}[a] (μM)	CDK1 IC_{50}[a] (μM)	VEGF-R2 IC_{50}[a] (μM)
1	CHO	79.9	0.076	63% at 100 μM	30% at 100 μM	<10% at 100 μM	<10% at 100 μM
7	CO_2H	75.2	0.041	0.776	73% at 100 μM	<10% at 100 μM	30% at 100 μM
6	CO_2Me	83.6	0.054	0.100	50% at 100 μM	ND	ND
8a	$CO_2CH_2CH_2$(morpholin-4- yl)	ND	0.150	1.53	55% at 100 μM	<10% at 100 μM	11% at 100 μM
8b	$CONHC_6H_5$	55.9	>100	>100	>100	>100	>100
8c	$CONHCH_2CH_2$(piperidin-1-yl)	42.4	ND	10.0	~100	>100	>100
8d	$CONHCH_2CH_2$(morpholin-4-yl)	35.3	ND	10.0	>100	>100	>100
8e	$CONHCH_2CH_2$(4-methoxyphenyl)	28.3	ND	>100	>100	>100	>100
8f	$CONHCH_2CH_2OMe$	36.6	ND	>100	>100	>100	>100
8g	$CON(CH_2CH_3)_2$	35.9	ND	>100	>100	>100	>100
8h	CO_2CH_2(3-fluorophenyl)	34.1	ND	>100	>100	>100	>100
8i	CONH(4-methoxyphenyl)	36.5	ND	>100	>10	>100	>100
8j	CN	88.5	0.027	0.470	33% at 100 μM	<10% at 100 μM	37% at 100 μM

[a] IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed. ND indicates that the IC_{50} experiment was not performed.

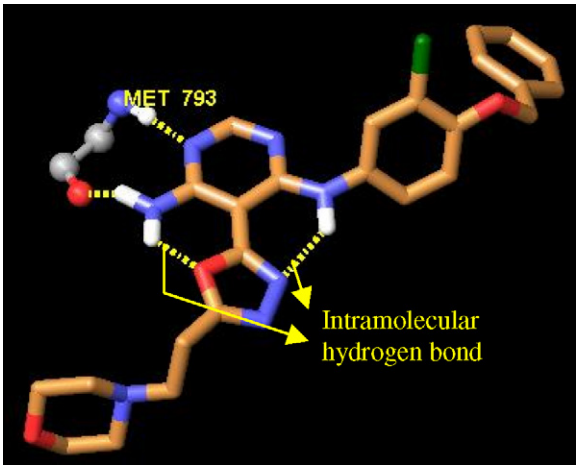
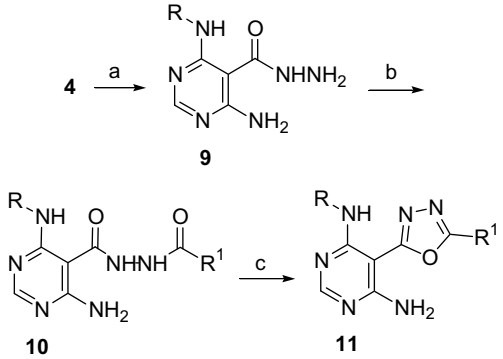


Figure 2. Docking of compound **11** with EGFR kinase shows intramolecular hydrogen bonds and intermolecular hydrogen bonds with Met793.

of compound **11** and NH_2 group of the amino-pyrimidine core. The second intramolecular hydrogen bond is between the N-3 of the oxadiazole ring and the NH of **11**.

Synthesis of the 1,3,4-oxadiazole analogue **11** is shown in Scheme 4. Methyl ester **6** was treated with hydrazine in ethanol to give the hydrazide **9**, which was coupled with 3-morpholin-4-yl-propionic acid using EDCI in DMF to give **10**. Dehydration of **10** with tosyl chloride and triethylamine in dichloromethane formed the oxadiazole ring **11** in excellent yield.[16]



Scheme 4. Reagents and conditions: (a) NH_2NH_2, EtOH, reflux, 58%; (b) EDCI, DMF, R^1CO_2H, 88%; (c) tosylchloride, Et_3N, DCM, 25 °C, 85% (R = 4-benzyloxy-3-chlorophenyl, R^1 = morpholin-4-yl-CH_2CH_2).

The EGFR and HER2 kinase data for analogues **9**–**11** are shown in Table 3. The kinase selectivity of hydrazide **9** favored HER2 inhibition with good HER2 kinase potency (IC_{50} = 146 nM) and moderate EGFR potency (IC_{50} = 458 nM). Surprisingly, the hydrazide **9** had better HER2 potency than any of the amides (Table 2, **8b**–**8g**, and **8i**). However, acylation of the hydrazide **9** afforded compound **10** that displayed a significant loss in HER2 potency (IC_{50} = 5.71 μM) accompanied by a mild loss in EGFR potency (IC_{50} = 620 nM). Cyclization of **10** to the oxadiazole **11** was accompanied by a 20-fold increase in EFGR inhibition (IC_{50} = 30 nM) and 70-fold increase in HER2 inhibition (IC_{50} = 80 nM). Notably, the isostere **11** was observed to be more active against EGFR (5-fold)

Table 3
Kinase activity for analogues **8a**, **9**–**11**

Compound	R	EGFR IC_{50}[a] (μM)	HER2 IC_{50}[a] (μM)	Aurora-A IC_{50}[a] (μM)	CDK1 IC_{50}[a] (μM)	VEGF-R2 IC_{50}[a] (μM)
8a	—	0.150	1.53	55% at 100 μM	<10% at 100 μM	11% at 100 μM
9		0.458	0.146	>10	>100	>10
10	Morpholin-4-yl-CH_2CH_2	0.620	5.70	>100	>100	>100
11	Morpholin-4-yl-CH_2CH_2	0.030	0.080	>100	>100	>100

[a] IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed. ND indicates that the IC_{50} experiment was not performed.

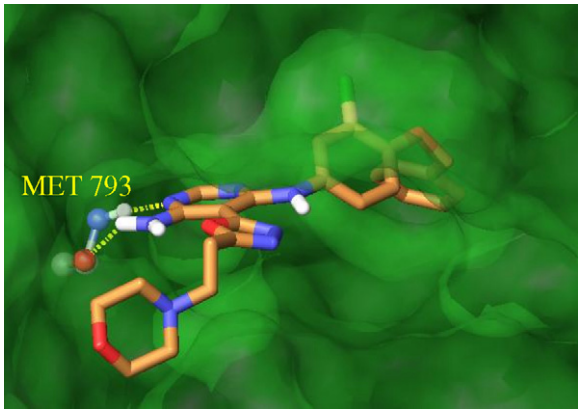


Figure 3. Compound **11** docked in to the ATP-site of EGFR1.

and HER2 (19-fold) than the ester **8a** after which it was designed. Additionally, **11** was highly selective for HER family kinases as demonstrated by its minimal activity against Aurora-A, CDK1, and VEGF-R2.

Compound **11** is potent inhibitor of the HER2 dependent cell lines N87 (IC_{50} = 411 nM), BT-474 (IC_{50} = 312 nM), and SK-BR3 (IC_{50} = 325 nM), but does not inhibit the growth of the non-HER2 dependent HeLa cell line (IC_{50} > 100 μM).

When modeled in EGFR kinase, **11** sits in the ATP-binding cleft. The amino-pyrimidine ring is hydrogen bonded to the hinge region between the NH_2- and COOH-terminal lobes of the kinase (Fig. 3). N-1 of the pyrimidine is hydrogen bonded to the main chain NH of Met793, whereas the amino group forms a hydrogen bond to the main chain C=O of Met793. The 3-chloro-4-(benzyloxy)aniline group is oriented deep in the ATP-binding site and makes predominantly hydrophobic interactions with the protein. The aniline nitrogen and the ether oxygen do not pick up any direct hydrogen bonding interactions with the protein. The 2-(morpholin-4-yl)ethylamino group on the oxadiazole ring is positioned toward the solvent interface, and provides a good handle to modify the physicochemical properties of the series.

In summary, we have described the early SAR leading to a novel 5-[1,3,4-oxadiazol-2-yl]-*N*-aryl-4,6-pyrimidine diamine, which represents a new and selective kinase inhibitor scaffold. The key modification that improved the potency of these analogues was the introduction of the 1,3,4-oxadiazole as an isosteric replacement of an ester functional group. This scaffold could be viewed as a mimic of the ubiquitous quinazoline scaffold for kinase inhibition. Extensive exploration of the SAR of this new scaffold and its ability to inhibit different kinases is underway.

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