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Synthesis and evaluation of 2,7-diamino-thiazolo[4,5-*d*] pyrimidine analogues as anti-tumor epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors

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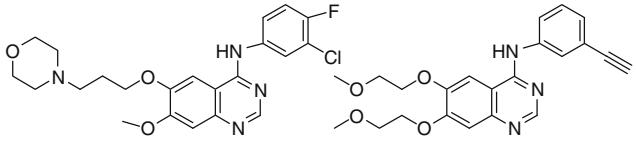
ABSTRACT

2,7-Diamino-thiazolo[4,5-*d*]pyrimidine analogues were synthesized as novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Representative compounds showed potent and selective EGFR inhibitory activities and inhibited in vitro cellular proliferation in EGFR-overexpressing human tumor cells. The synthesis and preliminary biological, physical, and pharmacokinetic evaluation of these thiazolopyrimidine compounds are reported.

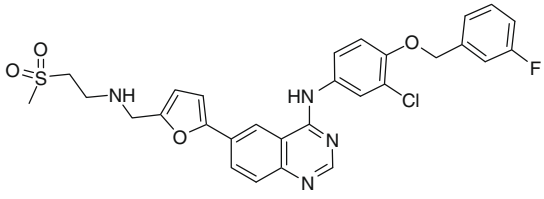
The epidermal growth factor receptor (EGFR) is a cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors. EGFR-dependent aberrant signaling is associated with cancer cell proliferation, apoptosis, angiogenesis, and metastasis. A number of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials.[1] For example, anilinoquinazoline-containing compounds gefitinib (Iressa™, **1**)[2] and erlotinib (Tarceva™, **2**)[3] have been developed and approved for the chemotherapeutic treatment of patients with advanced non-small-cell-lung cancer. The dual EGFR/HER2 inhibitor lapatinib (Tykerb™, also known as GW-572016) was recently approved for the treatment of HER2-positive metastatic breast cancer.[4] Many more compounds are still under evaluation in clinical trials for the treatment of cancer.[1]

In our effort to discover and develop protein kinase inhibitors as anti-cancer agents[5] we were interested to explore 2,7-diamino-thiazolo[4,5-*d*]pyrimidines as potential CDK (cyclin-dependent kinase) inhibitors.



Iressa, **1**

Tarceva, **2**



Lapatinib, **3**

These designed thiazolo[4,5-*d*]pyrimidines are related to 2,4-diaminothiazoles that have been previously reported to be potent inhibitors of both CDK[6,7] and GSK-3 (glycogen synthase kinase-3, which belongs to the same family of serine-threonine protein kinases as CDKs)[8] by different groups. For example, compound **4**, discovered by Agouron, has been shown to be a potent CDK inhibitor,[6] while compound **5** was disclosed by Novo Nordisk as a GSK-3 inhibitor.[8] In recognition of potential intra-molecular hydrogen bonding between the primary amino and carbonyl groups of compounds **4** and **5**, we proposed that bicyclic 2,7-diamino-thiazolo[4,5-*d*]pyrim-

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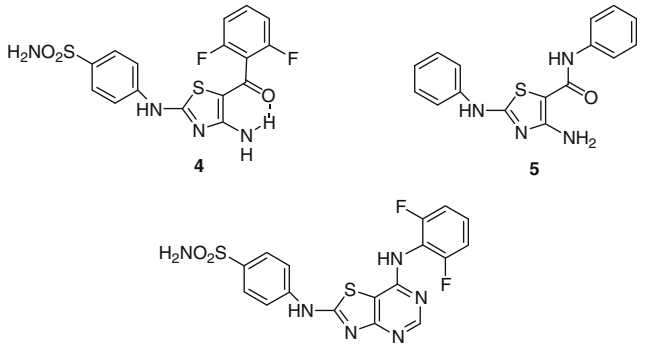
idines such as compound **6a** might be potential CDK inhibitors. Consequently, compound **6a** was synthesized and found to have a weak CDK1 inhibition with an IC_{50} of 19.8 μM. Subsequently, compound **6a** was found to have an IC_{50} of 0.187 μM against EGFR kinase, another kinase target screened in our oncology program.[9] This EGFR activity was not totally surprising as compound **6a** is structurally similar to the well-known anilinoquinazoline-containing EGFR inhibitors such as gefitinib, erlotinib, and lapatinib.

Based on this finding we synthesized and evaluated a series of thiazolo[4,5-*d*]pyrimidines with various structural modifications at the 2- and 7-positions, aimed at identifying potent, selective, and bioavailable EGFR inhibitors as anti-cancer agents. Herein we report their synthesis and preliminary biological evaluation.



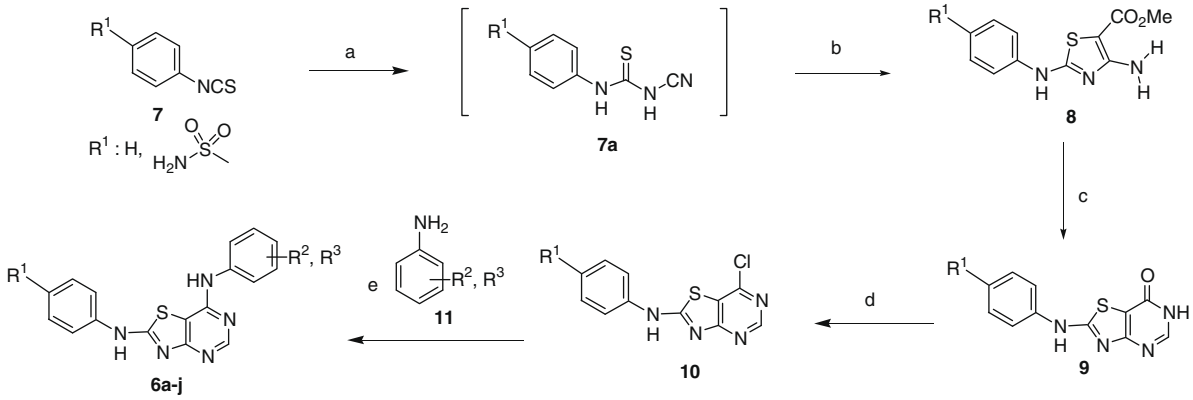
A general approach to synthesize the designed compounds **6a**–**j** is shown in Scheme 1, starting from commercially available isothiocyanates. The phenylisothiocyanates could also be prepared from substituted phenylamines and thiophosgene in the presence of concentrated hydrochloric acid. The reaction of phenylisothiocyanates **7** and cyanamide in the presence of a solution of sodium methoxide provided intermediates **7a**. The intermediates **7a** were reacted with methyl chloroacetate to provide substituted 4-amino-2-phenylamino-thiazole-5-carboxylic acid methyl esters **8**. Compounds **8** were treated with acetic anhydride in formamide to provide substituted 2-phenylamino-6*H*-thiazolo[4,5-*d*]pyrimidin-7-ones **9**. The chlorination of compounds **9** with $POCl_3$ in HMPA produced substituted (7-chloro-6,7-dihydro-thiazolo[4,5-*d*]pyrimidin-2-yl)-phenylamines **10**. The reaction of compounds **10** and substituted phenylamines **11** in a solvent such as isopropanol, diglyme, or butoxyethanol provided substituted N^2,N^7-diphenyl-thiazolo[4,5-*d*]pyrimidine-2,7-diamines **6a**–**j** in 20–65% yield.

An alternative general approach to synthesize the designed compounds **3** is shown in Scheme 2. Chloroacetonitrile was reacted with potassium methyl *N*-cyanodithioimidocarbonate **12**, followed by treatment with triethylamine to give the cyclic product 4-amino-2-methylsulfanyl-thiazole-5-carbonitrile **13**. Compound **13** upon heating with formic acid provided 2-methylsulfanyl-6*H*-thiazolo[4,5-*d*]pyrimidin-7-one **14**.[10] Chlorination of compound **14** with $POCl_3$ provided 7-chloro-2-methylsulfanyl-thiazolo[4,5-*d*]pyrimidine **15**. The reaction of compound **15** with substituted phenylamines **16,** upon heating at 140 °C in diglyme, provided substituted (3-phenyl)-(2-methylsulfanyl-thiazolo[4,5-*d*]pyrimidin-7-yl)-amines **17** in 53–92% yield. Compounds **17** were oxidized by MCPBA, followed by treatment with primary amines, **18** in glacial acetic acid to provide compounds **6k**–**v** in 55–78% yield.

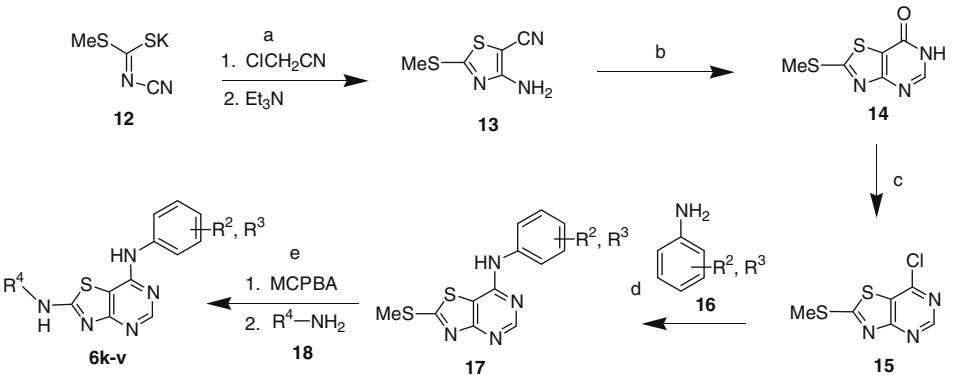
Table 1 shows the structures and EGFR inhibitory activities for the series of 2,7-diamino-thiazolo[4,5-*d*]pyrimidines. Structure–activity relationship (SAR) studies of the series were first focused on the 7-anilino substituent. 2,6-Difluoro substituents on the 7-anilino group of compound **6a** diminished potency compared to the unsubstituted compound **6b** (IC_{50} = 82 vs 187 nM). Small non-polar substituents at the meta position, such as chloro, bromo, or methyl, were preferred at the meta-position, as in compounds **6c**–**f** (IC_{50} = 7 to 14 nM). Introduction of a morpholino substituent to the 4-position of the anilino group (compounds **6g** and **6h**) drastically reduced EGFR potency (IC_{50} = 3.3 and 17.9 μM, respectively). Removal of the sulfonamide group of **6c** and **6e** also reduced EGFR potency by four- to sixfold (vs compound **6i** and **6j**, respectively). Likewise, introduction of solubilizing groups to the sulfamide group also reduced EGFR potency (compound **6k** vs compound **6e**, IC_{50} = 25 vs 9 nM).

SAR on the 2-anilino substituent was examined while retaining the favorable 4-fluoro-3-chloroanilino group at the 7-position of the scaffold. Replacement of the 2-anilino substituent with an aliphatic alkyl amino group (compound **6l**) substantially reduced EGFR potency (IC_{50} = 918 nM). However, unlike the 7-anilino substituent, the 2-anilino moiety tolerated substitution with solubilizing groups. Analogues with various dialkylaminomethyl (**6m**, **6o**, and **6q**–**s**) and dialkylaminoethyl (**6n**, **6p**) groups showed potent EGFR inhibition, with IC_{50} values ranging from 4 to 15 nM. Similarly, compound **6t** with a 4-(2-ethyl-imidazol-1-ylmethyl)-substituent was a potent EGFR inhibitor (IC_{50} = 6 nM).

Moving our attention back to the 7-position, replacement of the 4-fluoro-3-chloroanilino with 3-ethynyl-anilino, which is



Scheme 1. General synthetic approach to 2,7-diamino-thiazolo[4,5-*d*]pyrimidine analogues. Reagents and conditions: (a) cyanamide, sodium methoxide, rt, 3 h, 82%; (b) methyl chloroacetate, 50–60 °C, 12 h, 95%; (c) acetic anhydride, formamide, 150–180 °C, 7 h, 57–88%; (d) $POCl_3$ in HMPA, 70–80 °C, overnight, 53–69%; (e) 2-butoxyethanol, 150–180 °C, 4–7 h, 20–65%.



Scheme 2. Alternative synthetic approach to 2,7-diamino-thiazolo[4,5-*d*]pyrimidine analogues. Reagents and conditions: (a) (1) chloro acetonitrile, acetone, rt, 1 h; (2) triethylamine, rt, 72 h, 82%; (b) fomic acid, water, reflux, 4 h, 95%; (c) phosphorus oxytrichloride, reflux, 1 h, 57%; (d) substituted aniline, diglyme, 140 °C, 3–6 h, 53–92%; (e) (1) MCPBA, DCM, water, $NaHCO_3$, 5 °C, 2 h, (2) primary amine, 40 °C, 4 h, 55–78%.

Table 1
Structures and EGFR protein kinase inhibitory activity of the 2,7-diamino-thiazolo[4,5-*d*]pyrimidines

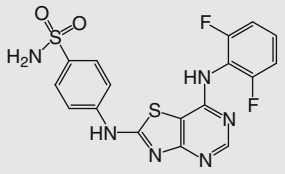
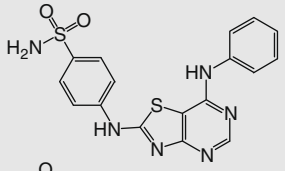
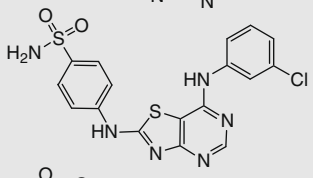
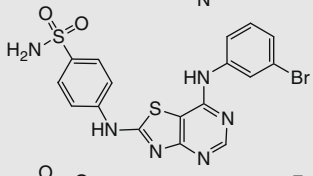
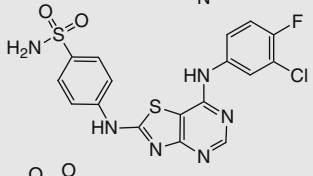
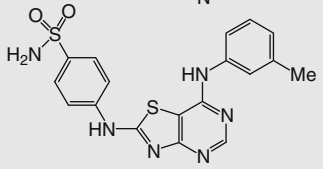
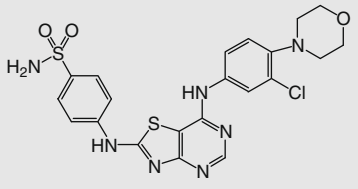
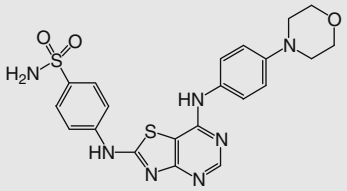
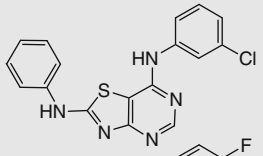
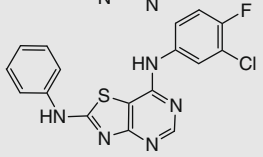
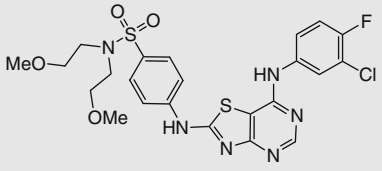
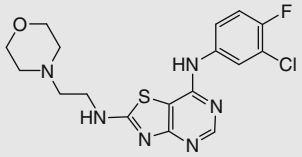
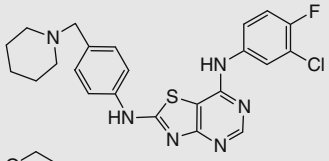
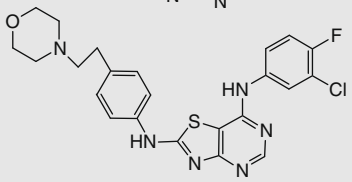
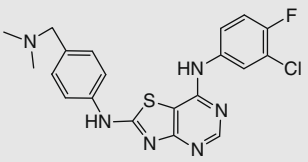
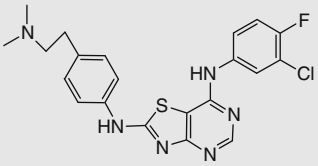
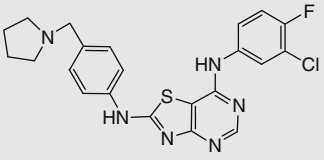
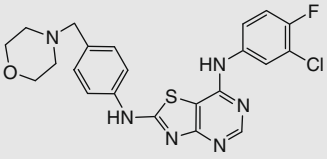
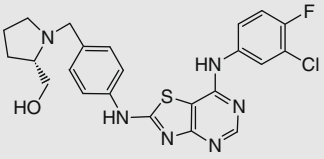
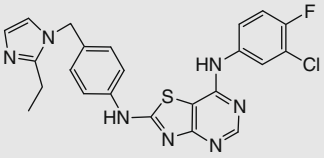
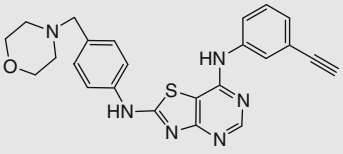
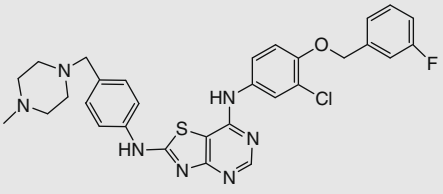
Compound	Structure	EGFR IC_{50} (nM)
6a		187
6b		82
6c		7
6d		10
6e		9
6f		14
6g		3300

Table 1 (*continued*)

Compound	Structure	EGFR IC_{50} (nM)
6h		17,900
6i		27
6j		59
6k		25
6l		918
6m		10
6n		15

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	EGFR IC_{50} (nM)
6o		12
6p		13
6q		4
6r		12
6s		11
6t		6
6u		43
6v		9

present in erlotinib, reduced EGFR potency (compound **6u** vs compound **6r**, IC_{50} = 43 vs 12 nM). On the other hand, replacement of the 4-fluoro-3-chloroanilino at the 7-position with 3-chloro-4-(3-fluoro-benzyloxy)-phenylamine group, which is present in lapatinib, caused no loss of EGFR potency (compound **6v**, IC_{50} = 9 nM).

Selected compounds proved to be active in vitro as anti-proliferatives in the human ovarian adenocarcinoma tumor cell line SK-OV-3. Representative EGFR inhibitors **6n**, **6o**, and **6r** showed potent inhibition of cellular proliferation with IC_{50} values below 1 μM against SK-OV-3 cell preparations (Table 2).

Table 2
Cell proliferation, HLM stability, solubility of lead 2,7-diamino-thiazolo[4,5-*d*]pyrimidine analogues

Compound	SK-OV-3 IC_{50} (μM)	HLM $t_{1/2}$ (min)	Solubility[a] (mg/mL)	
			pH 2.0	pH 7.4
6c	ND[b]	>100	<0.0002	<0.0002
6n	0.62	11	0.29	<0.0002
6o	0.56	>100	0.39	0.027
6r	0.57	19	>1.0	0.001
6s	ND	>100	0.36	<0.0002
6t	ND	3	0.30	<0.0002
19	ND	13	0.037	<0.0002

[a] The pH 2.0 buffer was prepared by adjusting the pH of a 0.05 M solution of NaH_2PO_4 containing 0.1 M NaCl to 2.0 with 85% phosphoric acid. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M solution of NaH_2PO_4 to pH 7.4 with 10 N NaOH.
[b] Not determined.

The lead compounds were also evaluated for stability in human liver microsomes (Table 2). Some compounds (**6c**, **6o**, and **6s**) had human liver microsome stability with $t_{1/2}$ > 100 min while others (**6n**, **6r**, and **6t**) had relatively low human liver microsome stability ($t_{1/2}$ 11, 19, and 3 min, respectively). The less stable compounds all had either a morpholine or imidazole substituent linked to the 2-anilino group via a short alkyl chain.

Solubilities of the lead compounds were measured in pH 2.0 and 7.4 aq buffer solutions (Table 2). Compounds with basic solubilizing groups (**6n**–**t**) were relatively soluble at pH 2.0 and usually much less soluble at pH 7.4. Compound **6c** without a solubilizing group was poorly soluble in both pHs.

Preliminary pharmacokinetic studies were conducted in rats with selected compounds (Table 3). Pharmacokinetic parameters were determined after single oral (10 mg/kg) and intravenous (2 mg/kg) dosing. Compound **6r** demonstrated fair oral bioavailability (20%) and exposure level (C_{max} and AUC) in rats while the other tested compounds had very low oral bioavailability. Interestingly, compound **6r** showed much better oral bioavailability in nude mice (67%) compared to rats. However, compound **6r** showed no in vivo anti-tumor efficacy in a preliminary tumor xenograft model in nude mice (unpublished results).

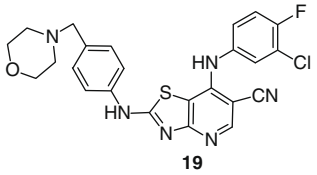
Extending our SAR study to the scaffold itself, structural modifications were made to replace the pyrimidine ring of the core thiazolo[4,5-*d*]pyrimidine with 4-cyanopyridine, in light of findings that quinoline-3-carbonitriles are bioisosteric with quinazolines in EGFR and Src tyrosine kinase inhibitors.[11] Thus, a new series of 2,7-di-substituted thiazolo[4,5-*b*]pyridine-6-carbonitriles as novel EGFR inhibitors has further been synthesized as reported previously.[12] Unfortunately, the new thiazolo[4,5-*b*]pyridine-6-carbonitrile series did not improve the EGFR potency, bioavailability, or other desirable properties, as shown in Tables 2 and 3 for representative compound **19**. Compound **19**, in comparison to its

Table 3
Pharmacokinetics properties of selected compounds[a]

Compound	*F* (%)	Cmax (PO) (ng/mL)	AUC (PO) (ng/mL h)	$T_{1/2}$ (PO) (h)
6c	0.8	39	224	ND[c]
6n	13	289	1174	1.8
6o	9	54	322	3.2
6r	20	527	2150	1.88
6r[b]	67	3850	10,838	2.9
6s	0.3	2.4	24	ND
19	10.8	28	184	4.9

[a] Compounds were dosed p.o. at 10 mg/kg in 0.5% methyl cellulose water and iv at 2 mg/kg in 10% Solutol® D5 W vehicle, respectively in rats except otherwise stated.
[b] Dosed in nude mice.
[c] Not determined.

counterpart **6r** in the thiazolo[4,5-*d*]pyrimidine series, had reduced EGFR potency (compound **19** vs compound **6r**, IC_{50} = 78 vs 12 nM); lower oral bioavailability in rats (compound **19** vs compound **6r**, 10.8% vs 20%); and lower aqueous solubility.



19

In summary, a series of 2,7-diamino-thiazolo[4,5-*d*]pyrimidines analogues showed modest to potent EGFR inhibition, with IC_{50} values ranging from micromolar to single digit nanomolar. SAR studies of the series revealed that the preferred substituent on the 7-anilino is a halogen such as chlorine or bromine at the meta-position, while the 2-anilino group tolerated substitution with various solubilizing groups. A subset of the selected compounds proved to be active in vitro as cellular anti-proliferatives in the EGFR-over-expressing human tumor cell line SK-OV-3. Representative compounds showed good aqueous solubility under acidic conditions and a mixed range of metabolic stabilities in human liver microsome preparations. Selected compounds were found to have low to modest oral bioavailability in rats. Further structural modification led to the discovery of 2,7-disubstituted 6-cyano-thiazolo[4,5-*b*]pyridines as EGFR inhibitors, although these novel kinase inhibitors offered no advantage over the 2,7-diamino-thiazolo[4,5-*d*]pyrimidines.

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References and notes

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