Journal of Medicinal Chemistry

Article

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Discovery of the Novel Potent and Selective FLT3 Inhibitor 1-{5-[7-(3-Morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]thiadiazol-2-yl}-3-*p*-tolylurea and Its Anti-Acute Myeloid Leukemia (AML) Activities *in Vitro* and *in Vivo*

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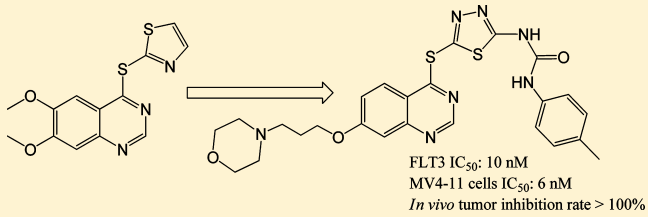
Supporting Information

ABSTRACT: Structure−activity relationship (SAR) studies of 2-(quinazolin-4-ylthio)thiazole derivatives, which are for optimizing the *in vitro* and *in vivo* antiacute myeloid leukemia (AML) activity of a previously identified FLT3 inhibitor 2-(6,7-dimethoxyquinazolin-4-ylthio)thiazole (**1**), are described. SAR studies centering around the head (thiazole) and tails (6- and 7-positions) of the quinazoline moiety of **1** led to the discovery of a series of compounds that exhibited significantly increased potency against FLT3-driven AML MV4-11 cells. Preliminary *in vivo* assays were carried out on three highly active compounds, whose results showed that 1-{5-[7-(3-morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]thiadiazol-2-yl}-3-*p*-tolylurea (**20c**) had the highest *in vivo* activity. Further *in vitro* and *in vivo* anti-AML studies were then performed on **20c**; in an MV4-11 xenograft mouse model, a once-daily dose of **20c** at 100 mg/kg for 18 days led to complete tumor regression without obvious toxicity. Western blot and immunohistochemical analysis were carried out to illustrate the mechanism of action of **20c**.



INTRODUCTION

FMS-like tyrosine kinase 3 (FLT3), a member of the class III receptor tyrosine kinase family, has been demonstrated to play an important role in the proliferation, differentiation, and apoptosis of hematopoietic cells.[1−3] Typically, FLT3 is activated upon the binding of the FLT3 ligand, which subsequently activates multiple downstream signaling pathways, including signal transducer and activator of transcription 5 (STAT5), Ras/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/AKT pathways. Activating mutations in FLT3 kinase are found in up to 1/3 of acute myeloid leukemia (AML) cases.[4,5] The most prevalent activating mutation is "internal tandem duplications" (ITD) in the juxtamembrane domain that leads to constitutive, ligand-independent activation of the kinase. The prognosis for AML patients with FLT3-ITD mutations is significantly worse than that for patients with wt-FLT3 (wild-type FLT3) when treated with standard therapy.[6−8] In addition, activating point mutations in the kinase activation loop of FLT3 are found in another subset of AML patients,[6,9−11] which are also associated with a poor prognosis for overall survival. Thus, FLT3 has been considered as a potential molecular target in the treatment of AML.

Because of the therapeutic values in AML, the discovery of FLT3 inhibitors has increasingly attracted much attention in recent years. To date, several FLT3 inhibitors including SU-5416,[12] SU-11248,[13] CHIR-258,[14] PKC-412,[15] CEP-701,[16] MLN-518,[17] BAY-43-9006,[18] KW-2449,[19] and AC220[20] have been advanced to clinical trials. However, except for very few of them, such as AC220, the clinical efficacy of most of these FLT3 inhibitors in patients with AML seems unimpressive, mainly because of their potency and/or adverse events that stem from their poor target selectivity. A survey analysis of FLT3 inhibitors that are currently in clinical trials made by us indicated that most of these inhibitors were not initially screened for sensitivity and selectivity against the FLT3 kinase but found an extra inhibitory potency against FLT3 in addition to the targeting of their designated kinases (see Table S1 in Supporting Information).[21−23] Therefore, the discovery and development of the next generation of novel FLT3 inhibitors with higher potency and better selectivity are strongly demanded at the present time.

Received: January 11, 2012
Published: March 27, 2012

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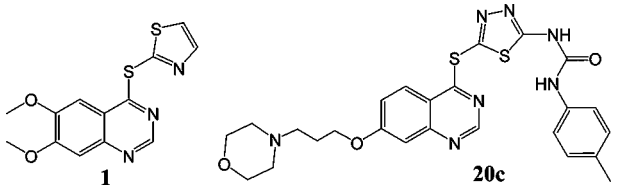
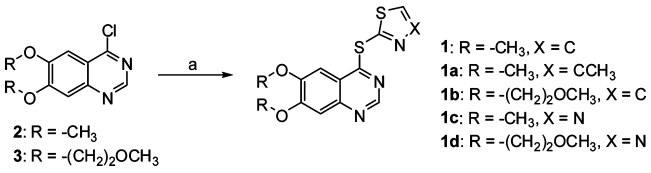
As a part of the efforts in discovering more potent and selective FLT3 inhibitors for the treatment of AML, we recently found a new lead compound, namely, 2-(6,7-dimethoxyquinazolin-4-ylthio)thiazole (**1**), with the aid of a restricted *de novo* design strategy proposed by us.[24] Compound **1** has a considerable inhibitory potency against FLT3 kinase with a half maximal inhibitory concentration (IC_{50}) of 0.007 μM. In addition, compound **1** also displayed a good selectivity for FLT3 against a panel of selected human kinases (see Table S2 in Supporting Information). Unfortunately, **1** showed poor antitumor activities *in vitro* and *in vivo* in models of FLT3-driven AML; the IC_{50} value against the human AML cell line MV4-11, which is a typical FLT3-ITD-expressing and a FLT3-driven AML cell line, is 1.14 μM, and the tumor inhibitory rate is less than 50% in its maximum dose in an MV4-11 xenograft model. The purpose here is to carry out a structural modification to optimize the *in vitro* and *in vivo* anti-AML activity of **1**. A series of novel 2-(quinazolin-4-ylthio)thiazole derivatives were synthesized and tested for their *in vitro* anti-AML activity. The most potent derivatives were then subjected to an *in vivo* assay in a mouse xenograft model of AML. Finally, a drug candidate, **20c**, which shows considerable *in vitro* and *in vivo* efficacy in models of FLT3-driven AML, was identified. In this account, we report the chemical synthesis, and the structure–activity relationship (SAR) of these novel series of compounds as well as *in vitro* and *in vivo* anti-AML activities of **20c**.



CHEMISTRY

As depicted in Scheme 1, compounds **1**, **1a–1d**, which have identical side chains at the C-6 and C-7 positions of the quinazoline, were prepared by treatment of the commercially available 4-chloro-6,7-dimethoxyquinazoline (**2**) or 4-chloro-6,7- bis(2-methoxyethoxy)quinazoline (**3**) with thiazole-2-thiol or its analogues ([1,3,4]thiadiazole-2-thiol and 4-methylthiazole-2-thiol).

Scheme 1[a]



[a]Reagents and conditions: (a) thiazole-2-thiol, [1,3,4]thiadiazole-2-thiol, or 4-methylthiazole-2-thiol, K_2CO_3, 2-butanone, 80 °C, 2–4 h.

Synthetic routes of 2-(quinazolin-4-ylthio)thiazole derivatives with nonidentical 6- and 7-position side chains are illustrated in Schemes 2 and 3. The production of 6-alkyloxy-7-methyoxyquinazoline analogues **8a–e** was started from the reaction of commercially available 7-methoxy-4-oxo-3,4-dihydroquinazolin-6-yl acetate (**4**) with phosphorus oxychloride in the presence of *N,N*-diethylaniline, which gave 4-chloro-7-methoxyquinazolin-6-yl acetate (**5**). Substitution of 4-chloride in **5** by thiazole-2-thiol in basic conditions afforded intermediate 7-methoxy-4-(thiazol-2-ylthio)quinazolin-6-yl acetate (**6**). Then intermediate **6** was cleanly hydrolyzed by $NH_3{\cdot}H_2O$ in methanol to obtain 7-methoxy-4-(thiazol-2-ylthio)quinazolin-6-ol (**7**), which was alkylated with various chloralkanes in the presence of K_2CO_3 to provide 6-alkyloxy-7-methyoxy analogues **8a–e**. The synthesis of 6-methoxy-7-alkyloxy analogues **13a–d** (Scheme 3) was commenced with commercially available 6-methoxy-4-oxo-3,4- dihydroquinazolin-7-yl acetate (**9**) using conditions similar to those for the preparation of **8a–e** to afford the desired compounds.

The synthesis of analogues **15a–o** with substitutive groups in the 4- and 5-positions of the thiazole ring is accomplished in a two-step process outlined in Scheme 4. Commercially available **2** and **3** were reacted with 2-(2-mercapto-4-methylthiazol-5-yl)acetic acid or 2-(2-mercaptothiazol-4-yl)acetic acid at reflux under basic conditions in butanone overnight to afford intermediates 4-substitutive-quinazolines **14a**, **14b**, and **14c**. Then, an amidation reaction of intermediates **14a**, **14b**, and **14c** with varieties of substitutive anilines was carried out in the presence of 1-hydroxybenzotriazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and *N,N*-diisopropylethylamine at reflux for 12–24 h in CH_2Cl_2 to provide the compounds shown in Table 2.

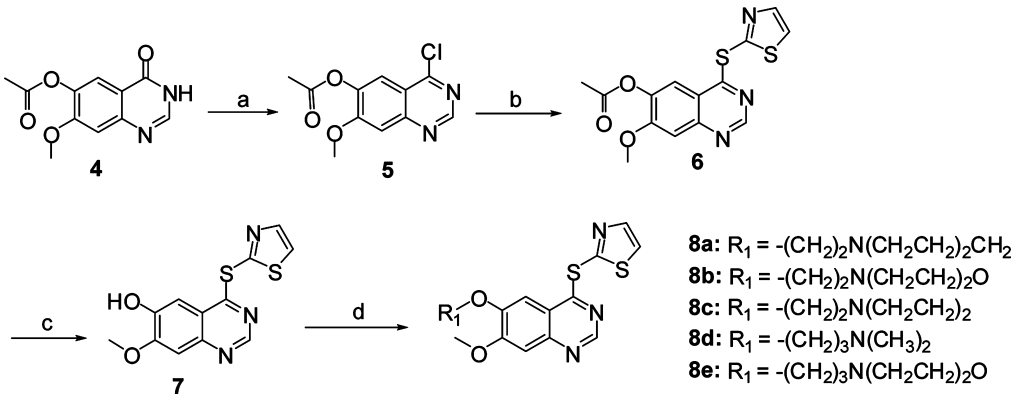
Finally, we synthesized a series of the 2-(quinazolin-4-ylthio)-[1,3,4]thiadiazole analogues **17a–h** and **20a–e** with the original thiazole ring replaced by the thiadiazole ring (see Schemes 5 and 6). The intermediate **16a** was achieved by reaction of 5-amino-[1,3,4]thiadiazole-2-thiol with acetic anhydride under reflux utilizing the synthetic method as described in ref 25. The intermediate **16b** was synthesized by an amidation reaction of 5-amino-[1,3,4]thiadiazole-2-thiol with acrylyl chloride in the presence of organic base using the synthetic procedure as described in ref 26. The intermediates **16c–i** were achieved by the reaction of 5-amino-[1,3,4]thiadiazole-2-thiol with substituent phenyl isocyanates under reflux in an appropriate solvent overnight using the methods described in refs 27 and 28. Then, intermediates **16a–h** were reacted with commercially available **3** in the presence of K_2CO_3 at 100 °C in *N,N*-dimethylformamide overnight to provide the 6,7-bis(2-methoxyethoxy) quinazoline analogues **17a–h** (see Scheme 5). The synthesis of 6-*H*-7-(3-morpholinopropoxy)quinazoline analogues **20a–e** was achieved via the intermediate **19**, which was synthesized starting from commercially available 7-fluoro-3*H*-quinazolin-4-one (**18**) (see Scheme 6). The fluoro group of 7-fluoro-3*H*-quinazolin-4-one was displaced with the anion of 3-morpholinopropan-1-ol at 100 °C in *N,N*-dimethylformamide overnight to give intermediate quinazolin-4-one (**19**). The intermediate **19** was then chlorinated by phosphorus oxychloride in the presence of *N,N*-diethylaniline followed by substitution with the intermediates **16b**, **c**, **g**, **f**, and **i** using the same conditions as those for the preparation of **17** described in Scheme 5 to afford the final products **20a–e**.

RESULTS AND DISCUSSION

Since our intention here is to optimize the functional activities of compound **1** both *in vitro* and *in vivo*, cell-based assays will be used to evaluate the bioactivity of synthesized compounds in the SAR studies.[29,30] Two cell lines including FLT3-driven MV4-11 and FLT3-independent HCT116 were chosen here;

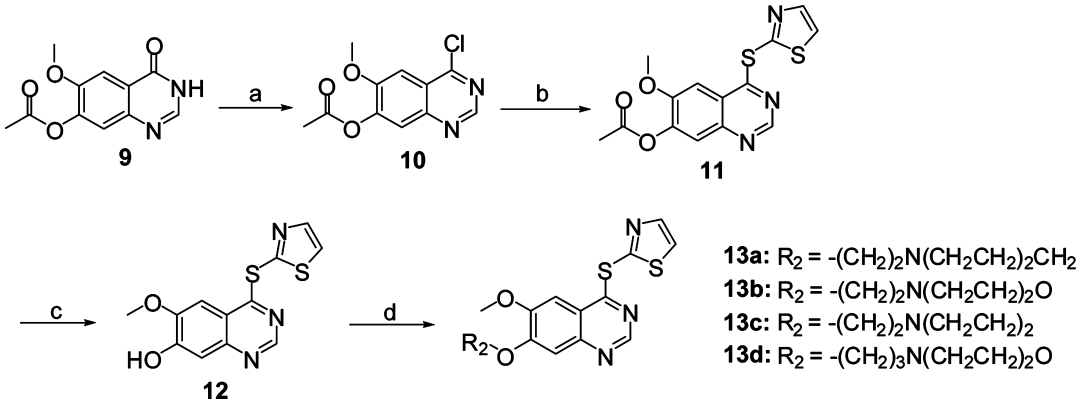
dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852–3866

Scheme 2[a]



[a]Reagents and conditions: (a) phosphorus oxychloride, toluene, *N*,*N*-diethylaniline, reflux, 3 h; (b) thiazole-2-thiol, K_2CO_3, 2-butanone, 80 °C, 2−4 h; (c) $NH_3{\cdot}H_2O$, MeOH, rt, 5 h; (d) alkyl halides, K_2CO_3, 2-butanone, 85 °C, 6 h.

Scheme 3[a]



[a]Reagents and conditions: (a) phosphorus oxychloride, chloroform, Et_3N, reflux, overnight; (b) thiazole-2-thiol, K_2CO_3, 2-butanone, 80 °C, 4 h; (c) $NH_3{\cdot}H_2O$, MeOH, rt, 5 h; (d) alkyl halides, K_2CO_3, 2-butanone, 85 °C, 6 h.

the involvement of HCT116 is for ruling out activity due to non-FLT3 mediated effects ("off target" or cellular toxic effects). Compounds are likely better FLT3 inhibitors if they have high potency against MV4-11 cells but low or no activity against HCT116. It should be pointed out from the start that the SAR obtained from such cell-based assays potentially report a combination of intrinsic activity of compounds against the kinase target modulated by the ability of compounds to permeate the cell wall during the assay. The enzymatic assays were also performed but just on compounds with higher potency at the cellular level. The most active compounds in both enzymatic and cellular levels will be selected for further *in vivo* experiments to evaluate their anti-AML activity.

Modifications of 6- and 7-Positions of Quinazoline. As previously indicated,[24] the 6- and 7-substituents of the quinazoline ring in **1** extend toward the solvent. The modifications of these positions were thus expected to help to improve the absorption ability of the cells.[29−32] Our attempts for the modifications of 6- and 7-substituents include increasing the chain length and varying the polarity of the tail. Various derivatives of **1** together with their bioactivities are presented in Table 1. Obviously, the modifications of 6- and 7-substituents of the quinazoline ring in **1** did not bring an increase in inhibitory activities against both MV4-11 and HCT116 cells. We thus started to modify the thiazole head.

Substitutions of 4- and 5-Positions of Thiazole. The modification toward the thiazole head was initiated to substitute the 4- and 5-positions of thiazole with different groups. At first, a single methyl group was introduced to the 4-position of thiazole. The resulting compound **1a** showed a similar antiproliferative activity against MV4-11 cells and no activity against HCT116 (unless it is necessary, we shall, later on, not mention the activity against HCT116 if there is no activity). We next explored the SAR of 5-position of thiazole; the methyl group was kept at the 4-position of thiazole due to the synthetic accessibility. The different substituents on the 5-position of thiazole (see **15a−15e**, Table 2) have considerable influence on the antiproliferative activity against MV4-11. The most active compound **15c**, in which the 5-position substituent is *N*-[3-(trifluoromethyl)phenyl]propionamide, has an IC_{50} value of 0.02 μM against MV4-11. The cellular activity increases more than 50 times relative to that of compound **1**. We then replaced the methoxy groups at the 6- and 7-positions of the quinazoline ring with longer methoxyethoxy groups, leading to compounds **15f−15k**. The bioactivities of the series of compounds were not improved, which is consistent with the above result that the modifications of 6- and 7-substituents of the quinazoline ring did not bring an increase in bioactivity. In the series of compounds, the most active compounds **15g** (IC_{50}: 0.025 μM) and **15h** (IC_{50}: 0.027 μM) again correspond to the 5-position substitution of *N*-[3-(trifluoromethyl)phenyl]-

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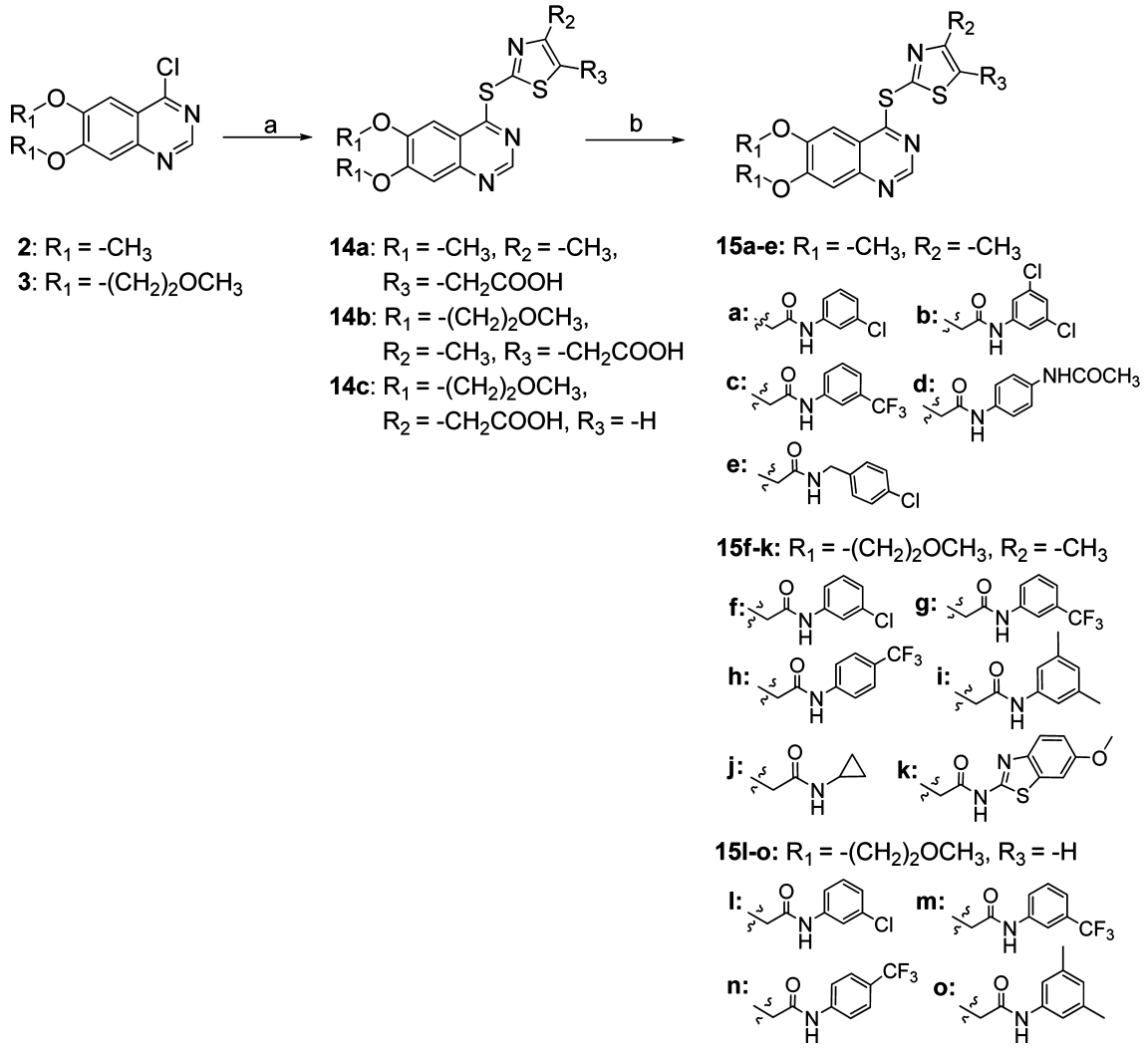
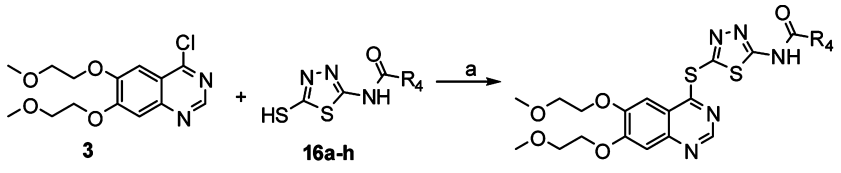
Scheme 4[a]



[a]Reagents and conditions: (a) 2-(2-mercapto-4-methylthiazol-5-yl)acetic acid or 2-(2-mercaptothiazol-4-yl)acetic acid, 2-butanone, K_2CO_3, reflux, overnight; (b) anilines or amines, HOBT, EDCI, DIEA, CH_2Cl_2, reflux, 12−24 h.

Scheme 5[a]



17a: R_4 = -methyl
17b: R_4 = -ethenyl
17c: R_4 = -NH-3-chlorophenyl
17d: R_4 = -NH-3-fluorophenyl
17e: R_4 = -NH-3-bromophenyl
17f: R_4 = -NH-3-acetylenephenyl
17g: R_4 = -NH-4-methylphenyl
17h: R_4 = -NH-4-trifluoromethylphenyl

[a]Reagents and conditions: (a) *N*,*N*-dimethylformamide, K_2CO_3, 100 °C, overnight.

propionamide (**15g**) and its isomer *N*-[4-(trifluoromethyl)-phenyl]propionamide (**15h**). Lastly, in order to explore the substitution effect of 4-position of thiazole, we synthesized compounds **15l−15o** that contain different substituents at the 4-position of thiazole. The bioactivity of **15l−15o** at the cellular level is clearly decreased relative to those of their counterparts **15f−15i** (see Table 2). From here, we can conclude that a bulkier substituent on the 5-position of thiazole is of benefit to increasing the bioactivity against MV4-11.

Replacement of Thiazole with Thiadiazole. In order to further improve the potency, we replaced the thiazole ring with the thiadizole ring based on the bioisosterism.[33] The first compound (**1c**) obtained contains a thiadizole head and two methyoxy groups at the 6- and 7-positions of quinazoline. Compound **1c** has FLT3 kinase inhibitory potency (IC_{50} = 0.009 μM) very similar to that of **1**. The antiproliferative activity (IC_{50}) of **1c** against MV4-11 is 1.20 μM, which is also similar to that of **1**. We then replaced methyoxy groups at the 6- and 7-positions of quinazoline with methoxyethoxy groups.

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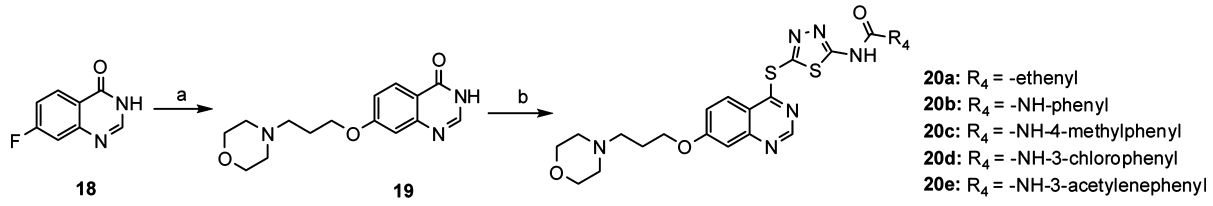
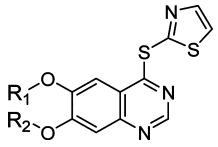
Scheme 6[a]



[a]Reagents and conditions: (a) 3-morpholinopropan-1-ol, NaH (60%), *N,N*-dimethylformamide, 100 °C, overnight; (b) (i) phosphorus oxychloride, *N,N*-diethylaniline, reflux, 3 h; (ii) **16**, *N,N*-dimethylformamide, K_2CO_3, 100 °C, overnight.

Table 1. 2-(Quinazolin-4-ylthio)thiazole Derivatives with Different Substituents at the 6- and 7-Positions of Quinazoline Together with Their Antiviability Activity against FLT3-ITD-Expressing MV4-11 Cells and FLT3-Independent HCT116 Cells



Cmpd.	R_1	R_2	MV4-11[a] (IC_{50}, μM)	HCT116 (IC_{50}, μM)
1	$-CH_3$	$-CH_3$	1.14±0.24	> 10
8a		$-CH_3$	1.61±0.71	> 10
8b		$-CH_3$	2.95±0.85	> 10
8c		$-CH_3$	1.24±0.33	> 10
8d		$-CH_3$	1.13±0.12	> 10
8e		$-CH_3$	1.02±0.02	> 10
13a	$-CH_3$		1.50±0.016	> 10
13b	$-CH_3$		3.00±0.78	> 10
13c	$-CH_3$		1.60±0.62	> 10
13d	$-CH_3$		1.15±0.13	> 10
1b	$-CH_2CH_2OCH_3$	$-CH_2CH_2OCH_3$	1.40±0.23	> 10

[a]Each compound was tested in triplicate; the data are presented as the mean ± SD.

The resulting compound **1d** has almost the same bioactivity as **1c**, which is again consistent with previous observations. We next optimized the thiadiazole head by replacing the 5-position with different substituents containing an amide or urea. We obtained a series of compounds **17a–h**, of which, **17f** that contains 3-ethynylphenylurea is the most active compound (IC_{50} = 0.005 μM). In order to further optimize the physicochemical properties, we introduced the polar group 4-(3-methoxypropyl) morpholine at the 7-position of quinazoline, which led to **20a–e** (see Table 3). Obviously, the introduction of a polar group considerably improves the bioactivity at the cellular level. Of special note are **20c** and **20e**, whose IC_{50} values against MV4-11 are 0.006 μM and 0.0003 μM, respectively (the kinase inhibitory potency against FLT3 is 0.01 μM for **20c** and 0.006 μM for **20e**, showing the consistency between cellular and enzymatic activities).

Kinase Inhibition Profile of 20c. The chemical modifications toward compound **1** resulted in three compounds (**17f**, **20c**, and **20e**), which potently inhibited the proliferation of AML MV4-11 cells with IC_{50} values less than 0.01 μM but displayed very weak or no inhibitory activity against HCT116, implying that these compounds could possibly have a potential value in the treatment of AML. Thus, the three compounds were selected to carry out preliminary *in vivo* anti-AML experiments using the MV4-11 tumor xenograft model. Compound **20c** displayed significant anti-AML potency (see Figure 2a), while **17f** and **20e** just showed very weak tumor inhibitory activity (the tumor inhibition rate is less than 50% at their maximum dose, namely, 50 mg/kg/d, for both of them by intraperitoneal administration). A plausible explanation for this is that **20c** has better bioavailability relative to **17f** and **20e**. Indeed, a comparison of pharmacokinetic profiles of **20c** and **20e** showed that **20c** has much better absorption than **20e** (C_{max} values are 5.31 μg/mL for **20c** and 2.74 μg/mL for **20e** at a dose of 40 mg/kg by intraperitoneal administration. For details, see Table S3 and Figure S1 in Supporting Information). Therefore, further studies on the kinase inhibition profile and anti-AML activity of **20c** both *in vitro* and *in vivo* will be carried out subsequently.

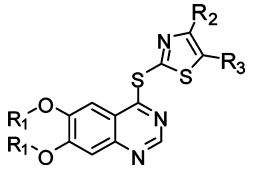
Kinase inhibition profiles of **20c** against a panel of recombinant human protein kinases are shown in Table 4. Compound **20c** potently inhibited FLT3 with an IC_{50} value of 0.01 μM (see Figure 1a). It just weakly inhibited Aurora A, FMS, FLT4, and c-Kit (IC_{50}s: 1.5 μM, 2.8 μM, 3.7 μM, and 6.8 μM, respectively). Compound **20c** displayed almost no inhibitory activity against the other 13 selected protein kinases. All of these demonstrate that **20c** is a potent FLT3 inhibitor with good kinase selectivity.

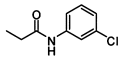
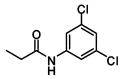
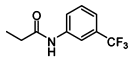
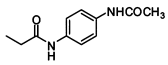
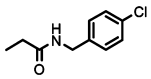
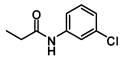
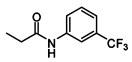
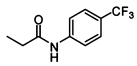
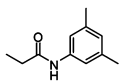
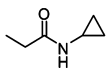
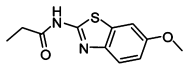
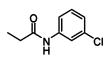
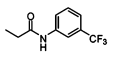
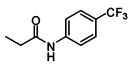
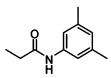
***In Vitro* Growth Inhibitory Activities of 20c against Leukemia and Other Cancer Cells.** The growth inhibitory potencies of **20c** against various cell lines, including leukemia and solid tumor cell lines, were examined, and the results are presented in Table 5. Compound **20c** potently inhibited the growth of MV4-11 cells that express FLT3-ITD, with an IC_{50} value of 0.006 μM. It just exhibited very weak inhibitory activity against human T lymphoma Jurkat cells, human Burkitt's lymphoma Ramos cells, human lung cancer PC-9 and H292 cells, and human epithelial carcinoma A431 cells (IC_{50}: 3.05 μM, 6.25 μM, 3.72 μM, 6.94 μM, and 8.91 μM, respectively). For other leukemia and solid tumor cell lines, including K562, U937, Karpas299, HCC827, A549, H2228, H820, MDA-MB-231, BT474, MCF-7, HCT116, SW480, LoVo, HeLa, SKOV-3, SK, DU145, PC-3, A431, and SH-SY5Y, **20c** did not exhibit an obvious growth inhibition effect at the concentration of 10 μM (Table 5).

Signaling Inhibition in Intact MV4-11 Cells. The ability of **20c** to inhibit the activation of FLT3 and downstream signaling proteins in intact cells was assessed using Western blot analysis. After a 5 h treatment with increasing

dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852–3866

Table 2. 2-(Quinazolin-4-ylthio)thiazole Derivatives with Different Substituents at the 4- and 5-Positions of the Thiazole Ring Together with Their Antiviability Activity against FLT3-ITD-Expressing MV4-11 Cells and FLT3-Independent HCT116 Cells



Cmpd.	R_1	R_2	R_3	MV4-11[a] (IC_{50}, μM)	HCT116 (IC_{50}, μM)
1a	$-CH_3$	$-CH_3$	H	3.68±0.79	> 10
15a	$-CH_3$	$-CH_3$		0.45±0.23	> 10
15b	$-CH_3$	$-CH_3$		1.40±0.11	> 10
15c	$-CH_3$	$-CH_3$		0.02±0.009	> 10
15d	$-CH_3$	$-CH_3$		> 5	> 10
15e	$-CH_3$	$-CH_3$		> 10	> 10
15f	$-CH_2CH_2OCH_3$	$-CH_3$		0.21±0.11	> 10
15g	$-CH_2CH_2OCH_3$	$-CH_3$		0.025±0.006	> 10
15h	$-CH_2CH_2OCH_3$	$-CH_3$		0.027±0.009	> 10
15i	$-CH_2CH_2OCH_3$	$-CH_3$		0.625±0.15	> 10
15j	$-CH_2CH_2OCH_3$	$-CH_3$		2.42±1.13	> 10
15k	$-CH_2CH_2OCH_3$	$-CH_3$		1.90±0.97	> 10
15l	$-CH_2CH_2OCH_3$		-H	3.20±1.12	> 10
15m	$-CH_2CH_2OCH_3$		-H	0.83±0.11	> 10
15n	$-CH_2CH_2OCH_3$		-H	2.88±0.45	> 10
15o	$-CH_2CH_2OCH_3$		-H	9.04±2.23	> 10

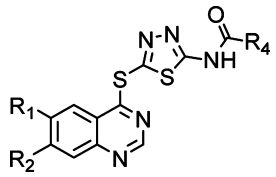
[a]Each compound was tested in triplicate; the data are presented as the mean ± SD.

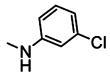
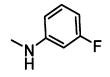
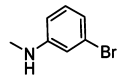
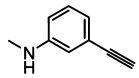
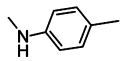
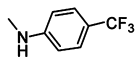
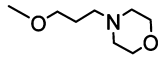
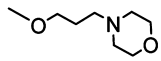
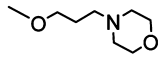
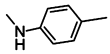
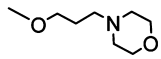
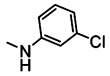
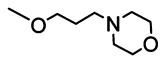
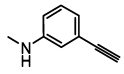
concentrations of **20c**, MV4-11 cells were harvested and lysed for an IP/wt assay. As shown in Figure 1b, **20c** inhibited FLT3 phosphorylation in a dose-dependent manner. Consistent with the downregulation of the phosphorylation of FLT3, the phosphorylation of the downstream signaling proteins STAT5 and ERK1/2 was also significantly inhibited at concentrations >0.1 μM (Figure 1b). We also observed that **20c** did not modulate the expression of these proteins during the drug treatment period.

***In Vivo* Effects of *20c* against s.c. MV4-11 Tumor Xenografts.** The *in vivo* antileukemia activity of **20c** was evaluated using an MV4-11 xenograft model. In the MV4-11 model, when the tumor grew to a volume of 300−500 mm^3, the mice were grouped and treated intraperitoneally once daily with

dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852−3866

Table 3. 2-(Quinazolin-4-ylthio)-1,3,4-thiadiazole Derivatives with Different Substituents at C-6 and C-7 of Quinazoline and the 5-Position of the Thiadiazole Ring Together with Their Anti-vViability Activity against FLT3-ITD-Expressing MV4-11 Cells and FLT3-Independent HCT116 Cells



Cmpd.	R_1	R_2	R_4	MV4-11[a] (IC_{50}, μM)	HCT116 (IC_{50}, μM)
1c	-	-	-	1.20±0.33	> 10
1d	-	-	-	1.35±0.58	> 10
17a	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$	-Me	> 11	> 10
17b	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$	$-CHCH_2$	> 10	> 10
17c	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.02±0.011	> 10
17d	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.40±0.18	> 10
17e	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.022±0.013	> 10
17f	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.005±0.002	> 10
17g	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.022±0.014	> 10
17h	$-OCH_2CH_2OCH_3$	$-OCH_2CH_2OCH_3$		0.30±0.16	> 10
20a	-H		$-CHCH_2$	6.00±1.25	> 10
20b	-H			0.01±0.006	> 10
20c	-H			0.006±0.003	> 10
20d	-H			0.012±0.005	> 10
20e	-H			0.0003±0.0001	> 10
Sunitinib				0.003±0.002	
MLN518				0.055±0.025	

[a]Each compound was tested in triplicate; the data are presented as the mean ± SD.

20, 40, or 100 mg/kg/d **20c** for 21 days. The tumor volumes were measured every 3 days. Treatment with **20c** at 100 mg/kg/d resulted in rapid and complete tumor regression in all mice of this group (see Figure 2a). Compound **20c** treatment at 20 mg/kg/d and 40 mg/kg/d significantly slowed down the tumor growth; the tumor inhibition rates are 66% and 84%, respectively. Moreover, during the whole experiment, no significant weight loss (see Figure 2b) or any other obvious signs of toxicity were observed for all of the **20c**-treated mice.

Compound **20c** was also evaluated for its effects on the tumor mitotic index (Ki67) and apoptosis using histological and immunohistochemical techniques. Similar to the tumor xenograft model, a dose of 20 mg/kg/d of **20c** was administered in the MV4-11 model. After treatment for 2 or 3 days, tumors were collected and analyzed. Tumor tissues

dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852−3866

Table 4. Kinase Inhibition Profile for 20c against Human FLT3 and a Panel of Other Selected Protein Kinases

kinase	IC_{50} (μM)
FLT3	0.01
c-Kit	6.8
FMS	2.8
PDGFR	>10
PDGFR	>10
FLT1	>1
KDR	>10
FLT4	3.7
Aurora A	1.5
IGF-1R	>10
Met	>10
Pim-1	>10
PLK1	>10
Ret	>10
Syk	>10
c-Raf	>10
EGFR	>10
JAK3	>10
Lck	>10

Table 5. Antiproliferative Activities of 20c against Various Cell Lines

cell lines	tumor type	IC_{50} (μM)
MV4−11	human acute myeloid leukemia	0.006
K562	human chronic myelogenous leukemia	12.65
U937	human leukemic monocyte lymphoma	>20
Jurkat	human T lymphoma	3.05
Ramos	human Burkitt's lymphoma	6.25
Karpas299	human anaplastic large cell lymphoma	>20
HCC827	human lung cancer	12.5
A549	human lung cancer	>20
H2228	human lung cancer	>20
H820	human lung cancer	>20
PC-9	human lung cancer	3.72
H292	human lung cancer	6.94
MDA-MB-231	human breast cancer	>20
BT474	human breast cancer	>20
MCF-7	human breast cancer	>20
HCT116	human colorectal carcinoma	>20
SW480	human colorectal carcinoma	11.16
LoVo	human colorectal carcinoma	>20
HeLa	human cervical carcinoma	>20
SKOV-3	human ovarian carcinoma	>20
SK	human ovarian carcinoma	>20
DU145	human prostate cancer	18.60
PC-3	human prostate cancer	15.72
A431	human epithelial carcinoma	8.91
SH-SY5Y	human neurobfastoma	>20

from the vehicle group were stained strongly with Ki67, indicating a large number of highly proliferative cells (see Figure 2c). Conversely, the tumor tissues from the **20c**-treated groups showed significantly fewer Ki67-positive cells. Furthermore, the TUNEL data showed an obvious increase in the percentage of apoptotic cells in a time-dependent manner. Hematoxylin staining also showed a significant regression of MV4-11 tumors (Figure 2c).

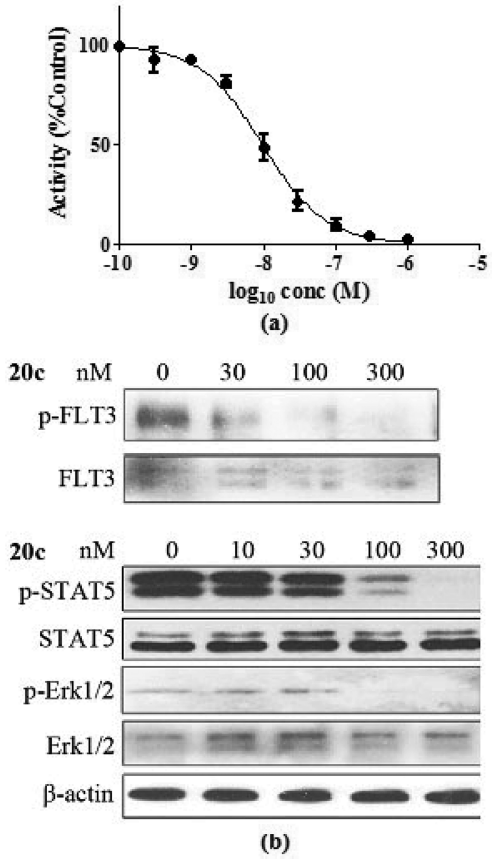


Figure 1. (a) Dose−response relationship of **20c** inhibition against the human FLT3 kinase (ATP concentration: 200 μM). (b) Western blot analysis showing that **20c** inhibits FLT3 autophosphorylation and the phosphorylation of downstream signaling proteins STAT5 and ERK1/2.

CONCLUSIONS

We have described the optimization of the FLT3 inhibitor 2-(6,7-dimethoxyquinazolin-4-ylthio)thiazole (**1**) that showed considerable enzymatic potency but very low anti-AML activity *in vitro* and *in vivo*. SAR studies centering around the head (thiazole) and tails (6- and 7-positions) of the quinazoline moiety led to the discovery of compounds displaying significantly increased potency against FLT3-driven MV4-11 cells but not active against HCT116 that is FLT3-independent. In-depth *in vitro* and *in vivo* assays were then carried out on one of the most active compounds, **20c**, which exhibited a higher potency against MV4-11 with IC_{50} = 0.006 μM and the maximum tumor inhibition rate in an *in vivo* MV4-11 xenograft model. Compound **20c** displayed very good FLT3 kinase selectivity against a panel of selected kinases and considerable sensitivity against FLT3-driven MV4-11 cells. Western blot and immunohistochemical analysis were carried out to illustrate the mechanism of action of **20c**, which showed that **20c** down-regulated the phosphorylation of FLT3/STAT5/ERK, blocked cell proliferation, and induced apoptosis in tumor tissue.

EXPERIMENTAL SECTION

Chemistry Methods. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The synthesis of compounds **1**, **8**, **13**, **15**, **17**, and **20** was as previously reported. All final compounds were purified to >95% purity, as determined by high-performance liquid chromatography

dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852−3866

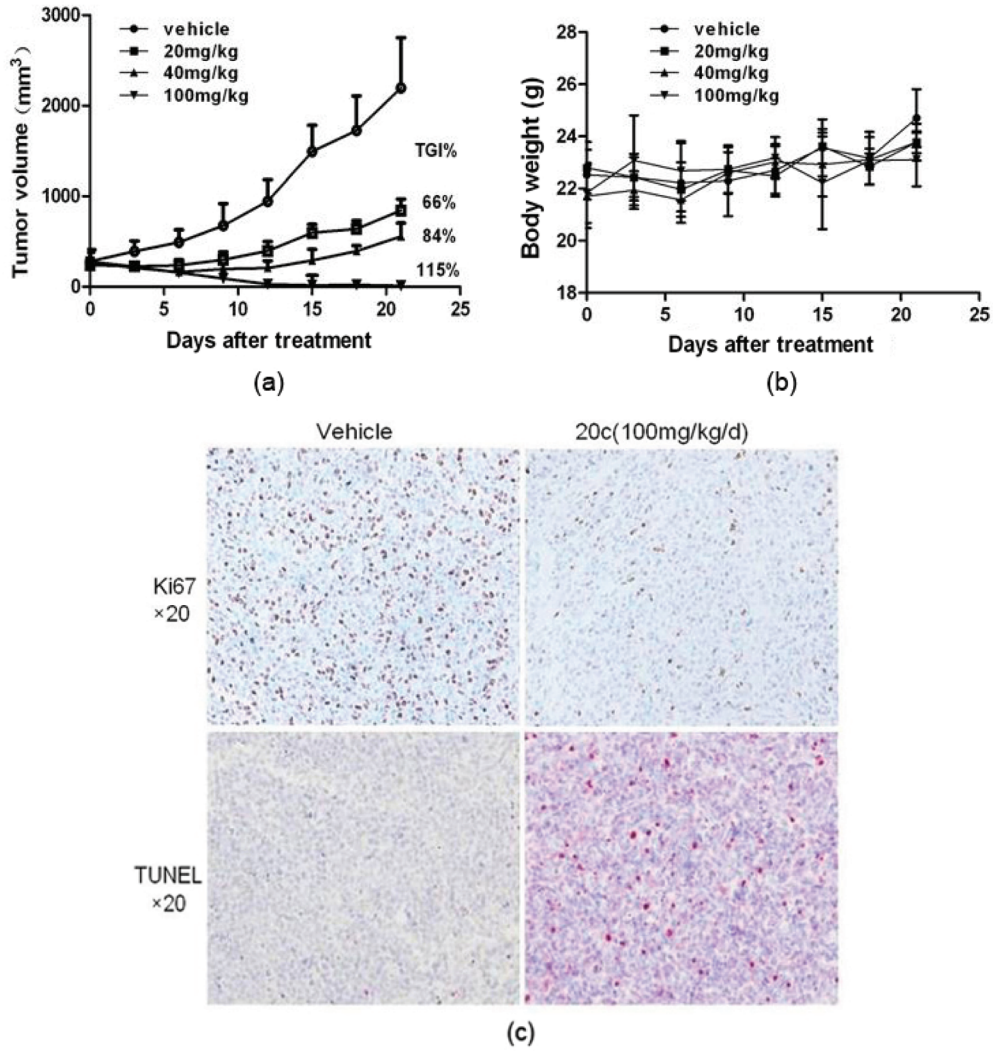


Figure 2. (a) Antitumor efficacy of **20c** at different doses in an *in vivo* MV4-11 xenograft model. (b) Average body weights for **20c**-treated mice groups at different doses (20 mg/kg, 40 mg/kg, and 100 mg/kg) and vehicle group in an *in vivo* MV4-11 xenograft model. (c) Ki67 and TUNEL staining of tumor tissues showing the inhibition of cell proliferation and the induction of apoptosis.

(HPLC). HPLC analysis was carried out on a Waters 2695 HPLC system with the use of a Kromasil C18 column (4.6 mm × 250 mm, 5 um). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. Multiplicities are given as s (singlet), d (doublet), dd (double−doublet), t (triplet), q (quadruplet), m (multiplet), and br s (broad signal). Low-resolution and high-resolution mass spectral (HRMS) data were obtained on an Agilent 1100 series LCMS with UV detection at 254 nm using electrospray ionization. Thin layer chromatography (TLC) was conducted on aluminum sheet silica gel Merck 60F254. The spots were visualized using ultraviolet light.

*6,7-Dimethoxy-4-(thiazol-2-ylthio)quinazoline (**1**).* A solution of 4-chloro-6,7-dimethoxy-quinazoline (**2**, 600 mg, 2.67 mmol), thiazole-2-thiol (330 mg, 2.8 mmol), and K_2CO_3 (1.1 g, 7.98 mmol) in 2-butanone was heated under reflux for 3 h. The reaction mixture was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 and water. The organic layer was collected, washed with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was decolorized by active carbon and purified by recrystallization from ethanol yielding 653 mg (80%, 99% HPLC purity) of **1** as fine white crystals. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88(s, 1H), 8.06(d, J = 3.2 Hz, 1H), 8.03(d, J = 3.2 Hz, 1H), 7.40(s, 1H), 7.29(s, 1H), 4.00(s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.4, 156.1, 153.8, 151.6, 150.5, 146.0, 143.0, 125.3, 117.4, 107.1, 100.7, 56.3, 56.1 ppm. MS (ESI, positive ion) m/z: 306.10 [M + H].

*6,7-Dimethoxy-4-(4-methylthiazol-2-ylthio)quinazoline (**1a**).* The title compound was prepared from **2** and 4-methylthiazole-2-thiol using the procedure previously described for compound **1** and was purified by column chromatography eluting with 30−50% EtOAc in petroleum ether to provide **1a** (300 mg, 82% yield, 98% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88(s, 1H), 7.29(s, 1H), 7.24(s, 1H), 7.15(d, J = 1.2 Hz, 1H), 4.05(s, 6H), 2.53(d, J = 0.8 Hz, 3H) ppm. MS (ESI, negative ion) m/z: 318.30 [M − H].

*6,7-Bis(2-methoxyethoxy)-4-(thiazol-2-ylthio)quinazoline (**1b**).* The title compound was prepared from **3** and thiazole-2-thiol using the procedure previously described for compound **1** and was purified by column chromatography eluting with 30−50% EtOAc in petroleum ether to provide **1b** (200 mg, 75% yield, 98% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89(s, 1H), 7.97(d, J = 3.2 Hz, 1H), 7.61(d, J = 3.2 Hz, 1H), 7.33(s, 1H), 4.33(m, 4H), 3.89(m, 4H), 3.50(d, J = 7.6 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{17}H_{19}N_3O_4S_2$ [M + H]$^+$ 394.0895; found, 393.8950.

*4-([1,3,4]Thiadiazol-2-ylthio)-6,7-dimethoxyquinazoline (**1c**).* The title compound was prepared from **2** and [1,3,4]thiadiazole-2-thiol using the procedure previously described for compound **1** and was purified by column chromatography eluting with 30−50% EtOAc in petroleum ether to provide **1c** (350 mg, 86% yield, 98% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.33(s, 1H), 8.97(s, 1H),

dx.doi.org/10.1021/jm300042x | J. Med. Chem. 2012, 55, 3852−3866

7.37(s, 1H), 7.23(s, 1H), 4.09(d, J = 5.6 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{12}H_{10}N_4O_2S_2$ [M + H]$^+$ 307.0323; found, 307.2012.

*4-([1,3,4]Thiadiazol-2-ylthio)-6,7-bis(2-methoxyethoxy)-quinazoline (**1d**).* The title compound was prepared from **3** and [1,3,4]thiadiazole-2-thiol using the procedure previously described for compound **1** and was purified by column chromatography eluting with 30−50% EtOAc in petroleum ether to provide **1d** (300 mg, 79% yield, 98% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86(s, 1H), 8.94(s, 1H), 7.46(s, 1H), 7.40(s, 1H), 4.38(q, 4H), 3.78(q, 4H), 3.37(d, J = 7.6 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{16}H_{18}N_4O_4S_2$ [M + Na]$^+$ 417.0667; found, 417.0803.

*7-Methoxy-6-[2-(piperidin-1-yl)ethoxy]-4-(thiazol-2-ylthio)-quinazoline (**8a**).* Phosphorus oxychloride (6.0 mL) was added to a solution of 7-methoxy-4-oxo-3,4-dihydro-quinazolin-6-yl acetate (**4**, 3.0 g, 12.8 mmol) and *N*,*N*-diethylaniline (2.5 mL, 15.4 mmol) in toluene at room temperature. Then the mixture was stirred in a preheated oil bath (100 °C) and refluxed for 3 h. The solvent was removed under reduced pressure. Ice water (50 mL) was added and the light brown precipitate filtered and washed with ice water (100 mL). The brownish precipitate was dried to give **5** (2.85 g, 88%). MS (ESI, positive ion) m/z: 253.03 [M + H].

A solution of **5** (2.0 g, 7.9 mmol), thiazole-2-thiol (927.6 mg, 7.9 mmol), and K_2CO_3 (3.27 g, 23.7 mmol) in 2-butanone was heated under reflux for 2 h. The reaction mixture was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 and water. The organic layer was collected, washed with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography eluting with 30−50% EtOAc in petroleum ether to provide **6** (2.35 g, 89% yield) as an orange yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95(s, 1H), 7.99(d, J = 3.2 Hz, 1H), 7.79(s, 1H), 7.63(d, J = 3.2 Hz, 1H), 7.43(s, 1H), 4.02(s, 3H), 2.41(s, 3H) ppm.

A solution of 7-methoxy-4-(thiazol-2-ylthio)quinazolin-6-yl acetate (**6**, 250 mg, 0.75 mmol) in $NH_3{\cdot}H_2O$/MeOH (10 mL, 1.5 N solution) was stirred at room temperature for 5 h. The reaction mixture was monitored by TLC. The solvent was evaporated under reduced pressure to give the crude intermediate **7** (yield by 100%). The residue was not further purified and directly used for the next step reaction.

A solution of 7-methoxy-4-(thiazol-2-ylthio)quinazolin-6-ol (**7**, 218.5 mg, 0.75 mmol) and K_2CO_3 (310.4 mg, 2.25 mmol) in 2-butanone (10 mL) was stirred at room temperature for 15 min, and then 2-piperidinoethylchloride hydrochloride (207 mg, 1.13 mmol) was added. This reaction mixture was heated under reflux for 7 h. The solvent was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 and water. The organic layer was collected, washed with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was recrystallized from ethyl ether yielding 153 mg (50%, 96% HPLC purity) of **8a** as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88(s, 1H), 8.06(d, J = 3.2 Hz, 1H), 8.03(d, J = 3.2 Hz, 1H), 7.40(s, 1H), 7.38(s, 1H), 4.31(t, J = 6.0 Hz, 2H), 4.00 (s, 3H), 2.75(t, J = 5.8 Hz, 2H), 2.48(br s, 3H), 1.84(s, 1H), 1.50−1.55(m, 4H), 1.39−1.40(m, 2H) ppm. MS (ESI, positive ion) m/z: 403.03 [M + H].

*4-Chloro-6-methoxyquinazolin-7-yl acetate (**10**).* Phosphorus oxychloride (3.0 mL) was added to a solution of 6-methoxy-4-oxo-3,4-dihydro-quinazolin-7-yl acetate (**9**, 3.0 g, 12.8 mmol) in chloroform at room temperature. Then the mixture was stirred in a preheated oil bath (70 °C) and refluxed for 1 h, and then triethylamine (4.8 mL) was added dropwise. The reaction mixture was refluxed for 12 h. The solvent was removed under reduced pressure. Ice water (50 mL) was added, and the aqueous solution was neutralized with saturated sodium carbonate solution. The aqueous solution was extracted with CH_2Cl_2. The organic layer was collected, washed with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to afford **10** (3.18 g, 98%) without further purification. MS (ESI, positive ion) m/z: 253.03 [M + H].

*7-Methoxy-6-(2-morpholinoethoxy)-4-(thiazol-2-ylthio)-quinazoline (**8b**).* The title compound was prepared from **7** and 4-(2-chloroethyl)morpholine hydrochloride using the procedure previously described for compound **8a** and was purified by recrystallization from ethyl ether. Yield: 100 mg (37%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88(s, 1H), 8.06(d, J = 3.6 Hz, 1H), 8.03(d, J = 3.2 Hz, 1H), 7.40(s, 1H), 7.36(s, 1H), 4.33(t, J = 5.8 Hz, 2H), 4.00(s, 3H), 3.61(t, J = 4.4 Hz, 4H), 2.80(t, J = 5.8 Hz, 2H), 2.51(t, J = 1.8 Hz, 4H) ppm. HRMS (m/z): calcd for $C_{18}H_{20}N_4O_3S_2$ [M + H]$^+$ 405.1055; found, 405.0109.

*7-Methoxy-6-(2-(pyrrolidin-1-yl)ethoxy)-4-(thiazol-2-ylthio)-quinazoline (**8c**).* The title compound was prepared from **7** and 1-(2-chloroethyl)pyrrolidine hydrochloride using the procedure previously described for compound **8c** and was purified by recrystallization from ethyl ether. Yield: 92 mg (42%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88(s, 1H), 8.06(d, J = 3.2 Hz, 1H), 8.03(d, J = 3.2 Hz, 1H), 7.41(s, 1H), 7.36(s, 1H), 4.31(t, J = 5.6 Hz, 2H), 4.01(s, 3H), 2.91(t, J = 5.6 Hz, 2H), 2.59(s, 4H), 1.71(s, 4H) ppm. MS (ESI, positive ion) m/z: 388.99 [M + H].

*3-[7-Methoxy-4-(thiazol-2-ylthio)quinazolin-6-yloxy]-N,N-dimethylpropan-1-amine (**8d**).* The title compound was prepared from **7** and 3-chloro-*N*,*N*-dimethylpropan-1-amine hydrochloride using the procedure previously described for compound **8d** and was purified by recrystallization from ethyl ether. Yield: 90 mg (46%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87(s, 1H), 8.06(d, J = 3.2 Hz, 1H), 8.03(d, J = 3.2 Hz, 1H), 7.39(s, 1H), 7.28(s, 1H), 4.22(t, J = 6.6 Hz, 2H), 4.01(s, 3H), 2.41(t, J = 7.0 Hz, 2H), 2.18(s, 6H), 1.96(m, 2H) ppm. MS (ESI, positive ion) m/z: 376.99 [M + H].

*7-Methoxy-6-(3-morpholinopropoxy)-4-(thiazol-2-ylthio)-quinazoline (**8e**).* The title compound was prepared from **7** and 4-(3-chloropropyl)morpholine using the procedure previously described for compound **8e** and was purified by recrystallization from ethyl ether. Yield: 150 mg (54%, 96% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90(s, 1H), 7.97(d, J = 3.2 Hz, 1H), 7.60(d, J = 3.6 Hz, 1H), 7.30(s, 1H), 4.26(t, J = 6.4 Hz, 2H), 4.04(s, 3H), 3.75(s, 4H), 2.60(br s, 2H), 2.51(br s, 4H), 2.15(br s, 2H) ppm. HRMS (m/z): calcd for $C_{19}H_{22}N_4O_3S_2$ [M + H]$^+$ 419.1212; found, 419.1217.

*6-Methoxy-7-[2-(piperidin-1-yl)ethoxy]-4-(thiazol-2-ylthio)-quinazoline (**13a**).* The title compound was prepared from **12** and 1-(2-chloroethyl)piperidine hydrochloride using the procedure previously described for compound **8a** and was purified by recrystallization from ethyl ether. Yield: 211 mg (36%, 96% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89(s, 1H), 7.97(d, J = 3.6 Hz, 1H), 7.60(d, J = 3.2 Hz, 1H), 7.30(d, J = 6.0 Hz, 2H), 4.34(br s, 2H), 4.04(s, 3H), 2.95(br s, 2H), 2.60(br s, 4H), 1.67(br s, 4H), 1.48(br s, 2H) ppm. HRMS (m/z): calcd for $C_{19}H_{22}N_4O_2S_2$ [M + H]$^+$ 403.1262; found, 403.1256.

*6-Methoxy-7-(2-morpholinoethoxy)-4-(thiazol-2-ylthio)-quinazoline (**13b**).* The title compound was prepared from **12** and 4-(2-chloroethyl)morpholine hydrochloride using the procedure previously described for compound **8a** and was purified by recrystallization from ethyl ether. Yield: 112 mg (29%, 96% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90(s, 1H), 7.97(d, J = 3.6 Hz, 1H), 7.60(d, J = 3.2 Hz, 1H), 7.30(d, J = 2.0 Hz, 2H), 4.35(t, J = 6.4 Hz, 2H), 4.04(s, 3H), 3.78(t, J = 4.4 Hz, 4H), 2.96(t, J = 5.8 Hz, 2H), 2.67(br s, 4H) ppm. HRMS (m/z): calcd for $C_{18}H_{20}N_4O_3S_2$ [M + H]$^+$ 405.1055; found, 405.1059.

*6-Methoxy-7-[2-(pyrrolidin-1-yl)ethoxy]-4-(thiazol-2-ylthio)-quinazoline (**13c**).* The title compound was prepared from **12** and 1-(2-chloroethyl)pyrrolidine hydrochloride using the procedure previously described for compound **8a** and was purified by recrystallization from ethyl ether. Yield: 124 mg (38%, 96% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89(s, 1H), 7.97(d, J = 3.6 Hz, 1H), 7.60(d, J = 3.2 Hz, 1H), 7.30(d, J = 2.8 Hz, 2H), 4.32(t, J = 6.0 Hz, 2H), 4.05(s, 3H), 3.07(t, J = 6.0 Hz, 2H), 2.70(br s, 4H), 1.85(br s, 4H) ppm. HRMS (m/z): calcd for $C_{18}H_{20}N_4O_2S_2$ [M + H]$^+$ 389.1106; found, 389.1101.

*6-Methoxy-7-(3-morpholinopropoxy)-4-(thiazol-2-ylthio)-quinazoline (**13d**).* The title compound was prepared from **12** and 4-(3-chloropropyl)morpholine using the procedure previously described for compound **8a** and was purified by recrystallization from ethyl ether. Yield: 150 mg (40%, 96% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90(s, 1H), 7.97(d, J = 3.2 Hz, 1H), 7.61(d, J = 3.2 Hz, 1H), 7.30(s, 1H), 7.27(s, 1H), 4.27(t, J = 6.6 Hz, 2H), 4.04(s, 3H), 3.76(br s, 4H), 2.62(br s, 2H), 2.53(br s, 4H), 2.16(br s, 2H) ppm.

HRMS (m/z): calcd for $C_{19}H_{22}N_4O_3S_2$ $[M + H]^+$ 419.1212; found, 419.1221.

*N-(3-Chlorophenyl)-2-[2-(6,7-dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]acetamide (**15a**).* A solution of 4-chloro-6,7-dimethoxyquinazoline (**2**, 500 mg, 2.22 mmol), 2-(2-mercapto-4-methylthiazol-5-yl)acetic acid (420 mg, 2.22 mmol), and K_2CO_3 (920 mg, 6.66 mmol) in 2-butanone was refluxed overnight. The reaction mixture was evaporated under reduced pressure. Ice water (100 mL) was added and the light yellow precipitate filtered and washed with ice water (100 mL) and CH_2Cl_2. The light yellow precipitate was dried to give **14a** (655 mg, 78%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.77(s, 1H), 8.87(s, 1H), 7.39(s, 1H), 7.28(s, 1H), 4.00(s, 6H), 3.92(s, 2H), 2.34(s, 3H) ppm.

2-[2-(6,7-Dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]-acetic acid **14a** (250 mg, 0.66 mmol) was reacted with 3-chlorobenzenamine (0.1 mL, 0.99 mmol) in the presence of 1-hydroxybenzotriazole (HOBT, 107.4 mg, 0.79 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 152.4 mg, 0.79 mmol), and *N,N*-diisopropylethylamine (DIEA, 0.13 mL, 0.79 mmol) in CH_2Cl_2 (10 mL). The mixture was heated under reflux for 24 h. Then, the mixture was cooled to room temperature, water (5 mL) added, and the precipitate filtered and washed with ice water and CH_2Cl_2 to obtain **15a** (194 mg, 60%). The light white precipitate was purified by recrystallization from ethanol (>98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52(s, 1H), 8.86(s, 1H), 7.82(s, 1H), 7.45(d, J = 8.4 Hz, 1H), 7.34−7.39(m, 2H), 7.29(s, 1H), 7.13(d, J = 7.6 Hz, 1H), 4.00(s, 8H), 2.39(s, 3H) ppm. MS (ESI, negative ion) m/z: 485.00 $[M - H]$.

*N-(3,5-Dichlorophenyl)-2-[2-(6,7-dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]acetamide (**15b**).* The title compound was prepared from **14a** and 3,5-dichlorobenzenamine using the procedure previously described for compound **15a** and was purified by recrystallization from ethanol. Yield: 110 mg (33%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.68(s, 1H), 8.87(s, 1H), 7.67(s, 2H), 7.40(s, 1H), 7.30(d, J = 8.4 Hz, 2H), 4.01(d, J = 7.6 Hz, 6H), 3.96(s, 2H), 2.39(s, 3H) ppm. MS (ESI, positive ion) m/z: 520.91 $[M + H]$.

*2-[2-(6,7-Dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]-N-[3-(trifluoromethyl)phenyl]acetamide (**15c**).* The title compound was prepared from **14a** and 3-(trifluoromethyl)benzenamine using the procedure previously described for compound **15a** and was purified by recrystallization from ethanol. Yield: 153 mg (45%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67(s, 1H), 8.87(s, 1H), 8.11(s, 1H), 7.78(d, J = 7.6 Hz, 1H), 7.58(t, J = 8.0 Hz, 1H), 7.40−7.44(m, 2H), 7.29(s, 1H), 4.04(s, 2H), 4.00(s, 6H), 2.40(s, 3H) ppm. MS (ESI, negative ion) m/z: 519.00 $[M - H]$.

*N-(4-Acetamidephenyl)-2-[2-(6,7-dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]acetamide (**15d**).* The title compound was prepared from **14a** and *N*-(4-aminophenyl)acetamide using the procedure previously described for compound **15a** and was purified by recrystallization from ethanol. Yield: 232 mg (57%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.27(s, 1H), 9.90(s, 1H), 8.86(s, 1H), 7.51(s, 4H), 7.39(s, 1H), 7.28(s, 1H), 4.00(s, 6H), 3.96(s, 2H), 2.40(s, 3H), 2.02(s, 3H) ppm. MS (ESI, negative ion) m/z: 508.00 $[M - H]$.

*N-(4-Chlorobenzyl)-2-[2-(6,7-dimethoxyquinazolin-4-ylthio)-4-methylthiazol-5-yl]acetamide (**15e**).* The title compound was prepared from **14a** and (4-chlorophenyl)methanamine using the procedure previously described for compound **15a** and was purified by recrystallization from ethanol. Yield: 153 mg (48%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85(s, 1H), 8.77(s, 1H), 7.39(d, J = 7.2 Hz, 3H), 7.30(d, J = 8.0 Hz, 3H), 4.30(d, J = 4.8 Hz, 2H), 4.00(s, 6H), 3.81(s, 2H), 2.35(s, 3H) ppm. MS (ESI, positive ion) m/z: 500.96 $[M + H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-(3-chlorophenyl)acetamide (**15f**).* A solution of 4-chloro-6,7-bis(2-methoxyethoxy) quinazoline (**3**, 500 mg, 1.60 mmol), 2-(2-mercapto-4-methylthiazol-5-yl)acetic acid (302.6 mg, 1.60 mmol), and K_2CO_3 (662 mg, 4.80 mmol) in 2-butanone was refluxed overnight. The reaction mixture was evaporated under reduced pressure. Ice water (100 mL) was added and the light yellow precipitate filtered and washed with ice water (100 mL). The light yellow precipitate was recrystallized from ethanol to give **14b** (655 mg, 78%).

2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}acetic acid (**14b**) (250 mg, 0.54 mmol) was reacted with the 3-chlorobenzenamine (0.1 mL, 0.80 mmol) in the presence of 1-hydroxybenzotriazole (HOBT, 87.0 mg, 0.65 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 123.4 mg, 0.65 mmol), and *N,N*-diisopropylethylamine (DIEA, 0.11 mL, 0.65 mmol) in CH_2Cl_2 (10 mL). The mixture was heated under reflux for 24 h. Then the clear solution was evaporated under reduced pressure, the residue was chromatographed on a silica gel column (eluting system: EtOAc) to obtain **15f** (200 mg, 62%) as a white powder, which was purified by recrystallization from ethanol (>98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52(s, 1H), 8.85(s, 1H), 7.82(t, J = 1.8 Hz, 1H), 7.42−7.46(m, 2H), 7.38(s, 1H), 7.34−7.36(m, 1H), 7.13(dd, J = 0.8 Hz, 0.8 Hz, 1H), 4.35(m, 4H), 4.01(s, 2H), 3.77(m, 4H), 3.36(d, J = 6.0 Hz, 6H), 2.39(s, 3H) ppm. MS (ESI, negative ion) m/z: 573.02 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-[3-(trifluoromethyl)phenyl]acetamide (**15g**).* The title compound was prepared from **14b** and 3-(trifluoromethyl)-benzenamine using the procedure previously described for compound **15f** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 119 mg (37%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67(s, 1H), 8.86(s, 1H), 8.11(s, 1H), 7.78(d, J = 8.0 Hz, 1H), 7.58(t, J = 8.0 Hz, 1H), 7.43(d, J = 6.8 Hz, 2H), 7.35(s, 1H), 4.36(t, J = 2.2 Hz, 4H), 4.03(s, 2H), 3.77(t, J = 2.2 Hz, 4H), 3.36(d, J = 5.6 Hz, 6H), 2.40(s, 3H) ppm. MS (ESI, negative ion) m/z: 607.22 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-[4-(trifluoromethyl)phenyl]acetamide (**15h**).* The title compound was prepared from **14b** and 4-(trifluoromethyl)-benzenamine using the procedure previously described for compound **15f** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 160 mg (49%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.69(s, 1H), 8.85(s, 1H), 7.82(d, J = 8.4 Hz, 2H), 7.70(d, J = 8.8 Hz, 2H), 7.42(s, 1H), 7.35(s, 1H), 4.36(m, 4H), 4.05(s, 2H), 3.77(m, 4H), 3.36(d, J = 6.0 Hz, 6H), 2.40(s, 3H) ppm. MS (ESI, negative ion) m/z: 607.22 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-(3,5-dimethylphenyl)acetamide (**15i**).* The title compound was prepared from **14b** and 3,5-dimethylbenzenamine using the procedure previously described for compound **15f** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 210 mg (69%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.17(s, 1H), 8.85(s, 1H), 7.42 (s, 2H), 7.35(s, 1H), 7.22(s, 1H), 6.71(s, 1H), 4.36(m, 4H), 3.96(s, 2H), 3.77(m, 4H), 3.36(d, J = 6.0 Hz, 6H), 2.39(s, 3H), 2.23(s, 6H) ppm. MS (ESI, negative ion) m/z: 567.02 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-cyclopropylacetamide (**15j**).* The title compound was prepared from **14b** and cyclopropanamine using the procedure previously described for compound **15f** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 90 mg (33%, > 98% HPLC purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88(s, 1H), 7.33(s, 1H), 7.30(s, 1H), 5.73(s, 1H), 4.32(t, J = 4.0 Hz, 4H), 3.89(t, J = 4.0 Hz, 4H), 3.71(s, 2H), 3.50(d, J = 6.4 Hz, 6H), 2.72(m, 1H), 2.42(s, 3H), 0.79(m, 2H), 0.50(m, 2H) ppm. MS (ESI, positive ion) m/z: 505.00 $[M + H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-4-methylthiazol-5-yl}-N-(6-methoxybenzo[d]thiazol-2-yl)acetamide (**15k**).* The title compound was prepared from **14b** and 6-methoxybenzo-[d]thiazol-2-amine using the procedure previously described for compound **15f** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 102 mg (30%, > 98% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60(s, 1H), 8.88(s, 1H), 7.66(d, J = 8.8 Hz, 1H), 7.58(s, 1H), 7.43(s,

1H), 7.35(s, 1H), 7.04(d, J = 7.2 Hz, 1H), 4.36(br s, 4H), 4.17(s, 2H), 3.81(s, 3H), 3.77(br s, 4H), 3.37(br s, 6H), 2.40(s, 3H) ppm. HRMS (m/z): calcd for $C_{28}H_{29}N_5O_6S_3$ $[M + H]^+$ 628.1358; found, 628.0308.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]thiazol-4-yl}-N-(3-chlorophenyl)acetamide (**15l**).* A solution of 4-chloro-6,7-bis(2-methoxyethoxy)quinazoline (**3**, 1.0 g, 3.20 mmol), 2-(2-mercaptothiazol-4-yl)acetic acid (561.0 mg, 3.20 mmol), and K_2CO_3 (1.32 g, 9.60 mmol) in 2-butanone was refluxed overnight. The reaction mixture was evaporated under reduced pressure. Ice water (100 mL) was added and the light yellow precipitate filtered and washed with ice water (100 mL). The light yellow precipitate was recrystallized from ethanol to give **14c** (1.1 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.53(s, 1H), 8.89(s, 1H), 7.77(s, 1H), 7.43(s, 1H), 7.34(s, 1H), 4.36(br s, 4H), 3.81(s, 2H), 3.78(br s, 4H), 3.36(br s, 6H) ppm. MS (ESI, negative ion) m/z: 450.10 $[M - H]$.

2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]thiazol-4-yl}-acetic acid (**14c**, 450.0 mg, 1.0 mmol) was reacted with the 3-chlorobenzenamine (0.2 mL, 1.6 mmol) in the presence of 1-hydroxybenzotriazole (HOBT, 162.2 mg, 1.2 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 230.0 mg, 1.2 mmol), and *N,N*-diisopropylethylamine (DIEA, 0.2 mL, 1.2 mmol) in CH_2Cl_2 (10 mL). The mixture was heated under reflux for 24 h. Then the clear solution was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column (eluting system: EtOAc) to obtain **15l** (360 mg, 64%, > 96% HPLC purity) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.47(s, 1H), 8.89(s, 1H), 7.85(s, 1H), 7.81(s, 1H), 7.48−7.44(m, 2H), 7.37−7.33(m, 2H), 7.12(d, J = 8.0 Hz, 1H), 4.36(br s, 4H), 3.91(s, 2H), 3.77(br s, 4H), 3.36(br s, 6H) ppm. MS (ESI, negative ion) m/z: 559.16 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]thiazol-4-yl}-N[3-(trifluoromethyl)phenyl]acetamide (**15m**).* The title compound was prepared from **14c** and 3-(trifluoromethyl)benzenamine using the procedure previously described for compound **15l** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 276 mg (47%, > 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62(s, 1H), 8.89(s, 1H), 8.14(s, 1H), 7.83(s, 1H), 7.79(d, J = 8.4 Hz, 1H), 7.57(t, J = 7.8 Hz, 1H), 7.44−7.41(m, 2H), 7.35(s, 1H), 4.36(br s, 4H), 3.93(s, 2H), 3.76(br s, 4H), 3.36(br s, 6H) ppm. MS (ESI, negative ion) m/z: 593.22 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]thiazol-4-yl}-N-[4-(trifluoromethyl)phenyl]acetamide (**15n**).* The title compound was prepared from **14c** and 4-(trifluoromethyl)benzenamine using the procedure previously described for compound **15l** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 334 mg (56%, > 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.64(s, 1H), 8.89(s, 1H), 7.83(d, J = 9.2 Hz, 3H), 7.69(d, J = 8.4 Hz, 2H), 7.44(s, 1H), 7.35(s, 1H), 4.36(br s, 4H), 3.94(s, 2H), 3.76(br s, 4H), 3.36(br s, 6H) ppm. MS (ESI, negative ion) m/z: 593.22 $[M - H]$.

*2-{2-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]thiazol-4-yl}-N-(3,5-dimethylphenyl)acetamide (**15o**).* The title compound was prepared from **14c** and 4-(trifluoromethyl)benzenamine using the procedure previously described for compound **15l** and was purified by column chromatography (eluting system: EtOAc) and recrystallization from ethanol. Yield: 355 mg (64%, > 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.10(s, 1H), 8.90(s, 1H), 7.79(s, 1H), 7.44(s, 1H), 7.36(s, 1H), 7.24(s, 2H), 6.70(s, 1H), 4.36(br s, 4H), 3.86(s, 2H), 3.76(br s, 4H), 3.36(br s, 6H), 2.23(s, 6H) ppm. MS (ESI, negative ion) m/z: 553.24 $[M - H]$.

*N-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}acetamide (**17a**).* A solution of 4-chloro-6,7-bis(2-methoxyethoxy)quinazoline (**3**, 300 mg, 0.96 mmol), *N*-(5-mercapto-[1,3,4]thiadiazol-2-yl)acetamide (**16a**, 185 mg, 1.05 mmol), and K_2CO_3 (397 mg, 2.88 mmol) in *N,N*-dimethylformamide (10 mL) was heated overnight at 100 °C. The reaction mixture was evaporated under reduced pressure. Ice water (20 mL) was added and the white precipitate filtered and washed with ice water (50 mL), which was recrystallized from ethanol to give **17a** (205 mg, 46%, 96% HPLC purity) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.82(s, 1H), 8.86(s, 1H), 7.44(s, 1H), 7.38(s, 1H), 4.38(br s, 4H), 3.78(br s, 4H), 3.37(d, J = 7.6 Hz, 6H), 2.24(s, 3H) ppm. HRMS (m/z): calcd for $C_{18}H_{21}N_5O_5S_2$ $[M + H]^+$ 452.1062; found, 452.1068.

*N-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}acrylamide (**17b**).* The title compound was prepared from **3** and *N*-(5-mercapto-[1,3,4]thiadiazol-2-yl)acrylamide (**16b**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 144 mg (31%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.07(s, 1H), 8.89(s, 1H), 7.46(s, 1H), 7.40(s, 1H), 6.60(dd, J = 10.0 Hz, 10.0 Hz, 1H), 6.49(d, J = 16.8 Hz, 1H), 6.02(d, J = 10.0 Hz, 1H), 4.38(br s, 4H), 3.78(br s, 4H), 3.36(d, J = 7.6 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{19}H_{21}N_5O_5S_2$ $[M + H]^+$ 464.1062; found, 464.1071.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-(3-chlorophenyl)urea (**17c**).* The title compound was prepared from **3** and 1-(3-chlorophenyl)-3-(5-mercapto-[1,3,4]-thiadiazol-2-yl)urea (**16c**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 440 mg (60%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41(s, 1H), 9.34(s, 1H), 8.89 (s, 1H), 7.73(s, 1H), 7.45(s, 1H), 7.38(br s, 3H), 7.14 (s, 1H), 4.38(m, 4H), 3.78(m, 4H), 3.37(d, J = 7.6 Hz, 6H) ppm. MS (ESI, negative ion) m/z: 563.00 $[M - H]$.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-(3-fluorophenyl)urea (**17d**).* The title compound was prepared from **3** and 1-(3-fluorophenyl)-3-(5-mercapto-[1,3,4]-thiadiazol-2-yl)urea (**16d**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 118 mg (46%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47(s, 1H), 9.66(s, 1H), 8.89(s, 1H), 7.35−7.51(m, 4H), 7.24(d, J = 8.0 Hz, 1H), 6.90(t, J = 7.6 Hz, 1H), 4.37(t, J = 4.2 Hz, 4H), 3.78(t, J = 6.2 Hz, 4H), 3.37(d, J = 7.2 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{23}H_{23}FN_6O_5S_2$ $[M + H]^+$ 547.1234; found, 547.1238.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-(3-bromophenyl)urea (**17e**).* The title compound was prepared from **3** and 1-(3-bromophenyl)-3-(5-mercapto-[1,3,4]-thiadiazol-2-yl)urea (**16e**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 188 mg (45%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60(s, 1H), 9.84(s, 1H), 8.90(s, 1H), 7.86(s, 1H), 7.20−7.48(m, 5H), 4.38(br s, 4H), 3.72(br s, 4H), 3.36(d, J = 7.6 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{23}H_{23}BrN_6O_5S_2$ $[M + H]^+$ 607.0433; found, 607.0424.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-(3-ethynylphenyl)urea (**17f**).* The title compound was prepared from **3** and 1-(3-ethynylphenyl)-3-(5-mercapto-[1,3,4]-thiadiazol-2-yl)urea (**16f**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 420 mg (67%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78(s, 1H), 9.47(s, 1H), 8.82(s, 1H), 7.80(s, 1H), 7.58(s, 1H), 7.24−7.43(m, 3H), 7.04(d, J = 5.6 Hz, 1H), 4.41(br s, 4H), 4.24(s, 1H), 3.77(br s, 4H), 3.36(d, J = 6.4 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{25}H_{24}N_6O_5S_2$ $[M + H]^+$ 553.1328; found, 553.1024.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-p-tolylurea (**17g**).* The title compound was prepared from **3** and 1-(5-mercapto-[1,3,4]thiadiazol-2-yl)-3-*p*-tolylurea (**16g**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 90 mg (42%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.21(s, 1H), 9.05(s, 1H), 8.88(s, 1H), 7.58(s, 1H), 7.45(s, 1H), 7.40(d, J = 7.6 Hz, 2H), 7.15(d, J = 8.4 Hz, 2H), 4.38(br s, 4H), 3.78(br s, 4H), 3.37(d, J = 7.6 Hz, 6H), 2.27(s, 3H) ppm. HRMS (m/z): calcd for $C_{24}H_{26}N_6O_5S_2$ $[M + H]^+$ 543.1484; found, 543.1220.

*1-{5-[6,7-Bis(2-methoxyethoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-[4-(trifluoromethyl)phenyl]urea (**17h**).* The title compound was prepared from **3** and 1-(5-mercapto-[1,3,4]thiadiazol-2-yl)-3-[4-(trifluoromethyl)phenyl]urea (**16h**) using the procedure previously described for compound **17a** and was purified by recrystallization from ethanol. Yield: 220 mg (40%, 96% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90(s, 1H), 10.89(s, 1H), 8.89(s, 1H), 7.71−7.74(m, 4H), 7.45(s, 1H), 7.39(s, 1H),

4.38(br s, 4H), 3.78(br s, 4H), 3.36(d, J = 7.2 Hz, 6H) ppm. HRMS (m/z): calcd for $C_{24}H_{23}F_3N_6O_5S_2$ [M + Na]$^+$ 619.1021; found, 619.1024.

*N-{5-[7-(3-Morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}acrylamide (**20a**).* To a *N,N*-dimethylformamide solution (5 mL) of 3-morpholinopropan-1-ol (2.5 mL, 18.3 mmol) at 0 °C was added 60% NaH (744 mg, 18.6 mmol), and the mixture was stirred for 30 min. To this cold solution was added a *N,N*-dimethylformamide solution (3 mL) of 7-fluoroquinazolin-4(3*H*)-one (**18**, 1.0 g, 6.1 mmol), and the mixture was heated at 100 °C overnight. The solvent was evaporated, and the residue was purified by ethyl ether to afford desired product **19** (1.43 g, 81%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11(s, 1H), 8.05(s, 1H), 8.00−8.02(m, 1H), 7.07−7.10(m, 2H), 4.15(t, J = 6.4 Hz, 2H), 3.58(t, J = 4.4 Hz, 4H), 2.43(t, J = 7.2 Hz, 2H), 2.37(br s, 4H), 1.92(m, 2H) ppm.

Phosphorus oxychloride (8.0 mL) was added dropwise to *N,N*-diethylaniline (1.0 mL) with magnetic stirring followed by the addition of 7-(3-morpholinopropoxy)quinazolin-4(3*H*)-one (**19**, 1.0 g, 3.45 mmol), and the reaction flask was immersed in a preheated oil bath (70 °C). The temperature was increased to 90 °C and kept at 90 °C for another 3 h. Most of the excess of phosphorus oxychloride was then removed under reduced pressure and the resulting dark oil triturated with toluene (3 × 40 mL). The residue was dissolved in water (20 mL) and extracted with EtOAc (2 × 15 mL). The pH of the aqueous phase was adjusted to 9−10 with Na_2CO_3, and it was extracted with CH_2Cl_2 (3 × 150 mL) and dried over Na_2SO_4, and the organic solvent was removed to afford the product as yellowish oil (900 mg, 84%) without further purification to use for the next step.

A solution of the yellowish oil (300 mg, 0.97 mmol), *N*-(5-mercapto-[1,3,4]thiadiazol-2-yl)acrylamide (**16b**, 220 mg, 1.18 mmol), and K_2CO_3 (404 mg, 2.93 mmol) in *N,N*-dimethylformamide (8 mL) was heated at 100 °C overnight. The reaction mixture was evaporated under reduced pressure. Ice water (20 mL) was added and the white precipitate filtered and washed with ice water (50 mL), which was recrystallized from ethanol to give **20a** (225 mg, 50%, 95% HPLC purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.02(s, 1H), 8.95(s, 1H), 8.15(d, J = 9.2 Hz, 1H), 6.60(dd, J = 10.0 Hz, 10.0 Hz, 1H), 6.49(dd, J = 1.6 Hz, 1.6 Hz, 1H), 6.02(dd, J = 2.0 Hz, 2.0 Hz, 1H), 4.27(t, J = 6.2 Hz, 2H), 3.59(t, J = 4.4 Hz, 4H), 2.48(br s, 2H), 2.40(br s, 4H), 1.97(m, 2H) ppm. HRMS (m/z): calcd for $C_{20}H_{22}N_6O_3S_2$ [M + H]$^+$ 459.1273; found, 459.1270.

*1-{5-[7-(3-Morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-phenylurea (**20b**).* The title compound was prepared from the intermediate yellowish oil and 1-(5-mercapto-[1,3,4]thiadiazol-2-yl)-3-phenylurea (**16i**) using the procedure previously described for compound **20a** and was purified by recrystallization from ethanol. Yield: 180 mg (70%, 95% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.43(s, 1H), 9.31(s, 1H), 8.94(s, 1H), 8.14(d, J = 9.2 Hz, 1H), 7.53(d, J = 8.0 Hz, 2H), 7.32−7.44(m, 4H), 7.07(t, J = 7.4 Hz, 1H), 4.27(t, J = 6.4 Hz, 2H), 3.59(t, J = 4.4 Hz, 4H), 2.48(br s, 2H), 2.42(br s, 4H), 1.98(m, 2H) ppm. HRMS (m/z): calcd for $C_{24}H_{25}N_7O_3S_2$ [M + H]$^+$ 524.1539; found, 524.1553.

*1-{5-[7-(3-Morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]-thiadiazol-2-yl}-3-p-tolylurea (**20c**).* The title compound was prepared from the intermediate yellowish oil and 1-(5-mercapto-[1,3,4]thiadiazol-2-yl)-3-*p*-tolylurea (**16g**) using the procedure previously described for compound **20a** and was purified by recrystallization from ethanol. Yield: 210 mg (59%, 95% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.40(s, 1H), 9.17(s, 1H), 8.95(s, 1H), 8.15(d, J = 9.2 Hz, 1H), 7.40−7.44(m, 4H), 7.15(d, J = 8.4 Hz, 2H), 4.27(t, J = 6.0 Hz, 2H), 3.60(br s, 4H), 2.48(br s, 2H), 2.42(br s, 4H), 2.27(s, 3H), 1.98(m, 2H) ppm. HRMS (m/z): calcd for $C_{25}H_{27}N_7O_3S_2$ [M + H]$^+$ 538.1695; found, 538.1685.

*1-(3-Chlorophenyl)-3-{5-[7-(3-morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]thiadiazol-2-yl}urea (**20d**).* The title compound was prepared from the intermediate yellowish oil and 1-(3-chlorophenyl)-3-(5-mercapto-[1,3,4]thiadiazol-2-yl)urea (**16c**) using the procedure previously described for compound **20a** and was purified by recrystallization from ethanol. Yield: 355 mg (72%, 95% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45(s, 1H), 10.19(s, 1H), 8.95(s, 1H), 8.15(d, J = 8.8 Hz, 1H), 7.77(s, 1H), 7.34−7.44(m, 4H), 7.10(d, J = 7.2 Hz, 1H), 4.27(t, J = 5.8 Hz, 2H), 3.61(br s, 4H), 2.55(br s, 2H), 2.48(br s, 4H), 2.00(m, 2H) ppm. HRMS (m/z): calcd for $C_{24}H_{24}ClN_7O_3S_2$ [M + H]$^+$ 558.1149; found, 558.1156.

*1-(3-Ethynylphenyl)-3-{5-[7-(3-morpholinopropoxy)quinazolin-4-ylthio]-[1,3,4]thiadiazol-2-yl}urea (**20e**).* The title compound was prepared from the intermediate yellowish oil and 1-(3-ethynylphenyl)-3-(5-mercapto-[1,3,4]thiadiazol-2-yl)urea (**16f**) using the procedure previously described for compound **20a** and was purified by recrystallization from ethanol. Yield: 368 mg (77%, 95% HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.85(s, 1H), 9.83(s, 1H), 8.94(s, 1H), 8.14(d, J = 8.8 Hz, 1H), 7.57(d, J = 8.0 Hz, 1H), 7.32−7.43(m, 3H), 7.15(d, J = 7.6 Hz, 2H), 4.26(t, J = 6.0 Hz, 2H), 4.19(s, 1H), 3.59(br s, 4H), 2.47(br s, 2H), 2.41(br s, 4H), 1.97(m, 2H) ppm. HRMS (m/z): calcd for $C_{26}H_{25}N_7O_3S_2$ [M + H]$^+$ 548.1539; found, 548.1553.

Kinase Inhibitory Assays. The kinase inhibitory assays were performed according to the KinaseProfiler assay protocols of Upstate Biotechnology (Millipore).

Cell Culture. Unless specifically mentioned, the cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in RPMI 1640 or DMEM culture medium containing 10% fetal bovine serum (v/v) in 5% CO_2 at 37 °C, except for MV4-11 cells, which were cultured in IMDM culture medium.

Cell Viability Assays. The viability of cells was determined using the MTT assay method. The leukemia cells were seeded in a 96-well plate at 1−4 × 10^4 cells per well, and an equal volume of medium containing increasing concentrations of inhibitors was added to each well. At the end of the incubation period (72 h at 37 °C), 20 μL of 5 mg/mL MTT reagent was added per well for 2−4 h of incubation, and 50 μL of 20% acidified SDS per well was used to lyse the cells. The other cell lines were seeded in 96-well plates at a density of 2−5 × 10^3 cells/well for 24 h followed by replacement of the medium with serial dilutions of inhibitors in culture medium. Following a 72-h incubation, the MTT reagent was added for a 2−4-h incubation, and 100% DMSO was used to dissolve the cells. Finally, the light absorption (OD) of the dissolved cells was measured at 570 nm using a SpectraMAX M5 microplate spectrophotometer (Molecular Devices). All experiments were performed in triplicate. The percentage of viability was calculated and compared with that of the control cells treated with DMSO (0.1%).

Immunoprecipitation and Western Blot Assays. Immunoprecipitation and immunoblotting were performed for the analysis of FLT3 autophosphorylation. After treatment with a series of concentrations of **20c** for 24 h at 37 °C, MV4-11 cells were harvested, washed in ice-cold PBS, and lysed with RIPA buffer (10 mM Tris-HCl (pH 7.8), 1% NP40, 0.15 M NaCl, 1 mM EDTA, 10 μM aprotinin, 1 mM NaF, and 1 mM Na_3VO_4). Samples were incubated overnight at 4 °C with the anti-FLT3 antibody, and immune complexes were precipitated with protein A agarose (Roche Applied Science) at 4 °C. The precipitated samples were subjected to immunoblot analysis to detect FLT3 phosphorylation by probing with the antiphosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY). Total FLT3 was measured with an anti-FLT3 antibody after the PVDF membrane had been incubated with stripping buffer. STAT5 and Erk1/2 phosphorylation was detected through immunoblot analysis. Briefly, after 20 h of **20c** treatment, MV4-11 cells were harvested and lysed with RIPA buffer. Samples were analyzed by immunoblotting as outlined above using antiphospho-STAT5, anti-STAT5, anti-p44/42 MAPK, and antiphospho-p44/42 MAPK antibodies.

***In Vivo* Models.** MV4-11 cells were harvested during the exponential-growth phase, washed twice with serum-free medium, and resuspended at the concentration of 1 × 10^8/mL. One hundred microliters of cell suspensions was injected subcutaneously into the hind flank of each female NOD-SCID mouse (6−7 weeks old). The tumors were allowed to grow to 300−500 mm^3 at which point the mice were randomized into 4 groups (6 mice for each group) and dosed with **20c** (20, 40, or 100 mg/kg/d) or vehicle. The compounds

were dissolved in 25% (v/v) PEG400 plus 5% DMSO and administered intraperitoneally at a dose of 5 mL/kg. Tumor growth was measured every 3 days using Vernier calipers for the duration of the treatment. The volume was calculated as follows: tumor volume = $a \times b^2/2$ (a, long diameter; b, short diameter).

Histopathology and IHC. NOD-SCID mice bearing tumors or having undergone bone marrow engraftment were treated with **20c** at a dose of 20 mg/kg/d (ip). At the indicated time after dosing, individual mice were sacrificed. The tumors or femoral bones were fixed with formalin and embedded in paraffin (femoral bones were decalcified). Sections measuring 4−8 μm in thickness were prepared for histological and immunohistochemical analysis. Proliferation was detected using immunostaining with the Ki67 antibody (Thermo Fisher Scientific, Fremont, CA). Apoptosis was determined using transferase-mediated dUTP nick-end labeling (TUNEL) and staining (Roche Applied Science). Finally, images were captured with an Olympus digital camera attached to a light microscope.

Pharmacokinetic Analysis. Male Sprague−Dawley rats (200−250 g) were used and randomly divided into two groups (n = 3 in each group). A catheter was surgically placed into the femoral vein for collection of blood samples. Rats were fasted overnight before dosing. Compounds **20c** and **20e** were administered intraperitoneally (ip) at a dose of 40 mg/kg. Plasma concentrations of each compound were determined by LC-MS/MS (a 3200 QTRAP system, Applied Biosystems/MDS Sciex).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IC_{50} values of **1** against human FLT3 and pharmacokinetic properties of **20c** and **20e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (81172987), the National S&T Major Project (2012ZX09102-101-002), and the 863 Hi-Tech Program (2012AA020301 and 2012AA020308).

■ ABBREVIATIONS USED

FLT3, FMS-like tyrosine kinase 3; STAT5, signal transducer and activator of transcription 5; AML, acute myeloid leukemia; SAR, structure−activity relationship; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; ITD, internal tandem duplications; TLC, thin layer chromatography; HOBT, 1-hydroxybenzotriazole; EDCI, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride; DIEA, *N*,*N*-diiso-propylethylamine

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