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Synthesis and c-Met Kinase Inhibition of 3,5-Disubstituted and 3,5,7-Trisubstituted Quinolines: Identification of 3-(4-Acetylpiperazin-1-yl)-5-(3-nitrobenzylamino)-7- (trifluoromethyl)quinoline as a Novel Anticancer Agent

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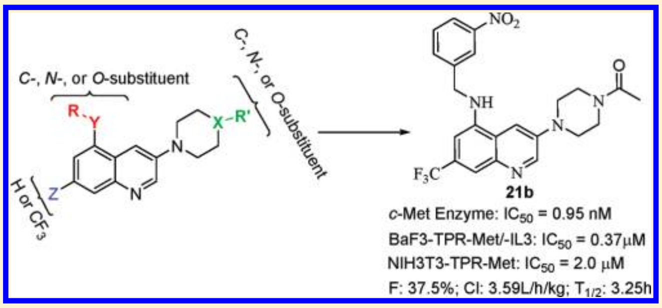
Supporting Information

ABSTRACT: By use of an improved synthetic strategy, a series of 3,5-disubstituted and 3,5,7-trisubstituted quinolines were readily prepared. 3,5,7-Trisubstituted quinolines **21a**−**c**, **21l**, and **27a**−**c** were identified as the most potent c-Met inhibitors with IC_{50} of less than 1.0 nM. Compound **21b** showed the most promising overall PK profile and has high potency and extraordinary selectivity to c-Met against c-Met family member Ron and 12 other tyrosine kinases. It produced constitutive inhibition of c-Met phosphorylation in c-Met dependent cell lines. At doses of 100 mg/kg, compound **21b** showed statistically significant tumor growth inhibition (68−69%) in both NIH-3T3-TPR-Met and U-87 MG human gliobastoma xenograft models. These results clearly indicated that compound **21b** is a potent and highly selective c-Met inhibitor. Its favorable in vitro and in vivo profiles warrant further investigation.



INTRODUCTION

c-Met is a unique receptor tyrosine kinase (RTK) structurally distinct from other RTK families. It is the cell surface receptor for hepatocyte growth factor (HGF) and is normally expressed by epithelial cells of many organs (liver, pancreas, prostate, kidney, muscle, and bone narrow) during embryogenesis and in adulthood.[1,2] The c-Met receptor, similar to its ligand HGF, consists of an entirely extracellular α-chain that is linked to a transmembrane β-subunit through a disulfide linkage. Binding of active HGF ligand to the c-Met extracellular domain causes receptor polymerization and phosphorylation of tyrosine residues in the intracellular c-Met domains.[3,4] Aberrant HGF/c-Met signaling has been identified in a wide range of human malignancies, including bladder, breast, cervical, colorectal, endometrial, gastric, kidney, liver, lung, pancreatic, prostate, and thyroid cancers.[5−11] This emphasizes c-Met as an attractive therapeutic target. More than 10 peptidomimetics or small-molecule c-Met inhibitors have presently been validated in the preclinical or clinical stages, exhibiting promising therapeutic benefits.

Several different strategies have been pursued to inhibit the c-Met pathway by blocking the interaction between c-Met and HGF with biological antagonists or neutralizing antibodies or by blocking the c-Met-dependent signaling via interfering with c-Met-associated signal transducers or downstream signaling components, as well as by blocking the c-Met catalytic activity with small-molecule inhibitors that compete for adenosine 5′-triphosphate (ATP) binding at the active site of the kinase.[12−21] Although much lagging behind the other strategies, the development of small molecular c-Met kinase inhibitors has made significant progress with several compounds reaching clinical trials recently.[14,15,18,22−24] These include the earlier identified [3-[[(*R*)-1-(2,6-dichloro-3-fluorophenyl)ethyl]oxy]-5-[1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-yl]amine[22] (**1**, PF-2341066), (3*Z*)-*N*-(3-chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-*N*-methyl-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonamide[23] (**2**, SU11274), and acylthiourea analogue **3**,[24] along with a large number of newly developed compounds bearing pyrrolotriazine, pyrrolopyridine, pyridopyrimidine, thienopyridine/pyrimidine, and triazolopyridazine frameworks as the primary pharmacophoric scaffolds.[12−21]

Through a high throughput screening (HTS) campaign, Porter et al.[25] recently reported two promising c-Met inhibitor hits featured by the existence of multisubstituted quinoxaline (**Ia**) and quinoline (**Ib**) cores, respectively (Figure 1). However, only the former structural core (quinoxalines **Ia**) was extensively explored leading to several compounds with two-digit nanomolar c-Met inhibitory potency. Although Porter and his colleagues also attempted to prepare 3,5-disubstituted or 3,5,7-trisubstituted

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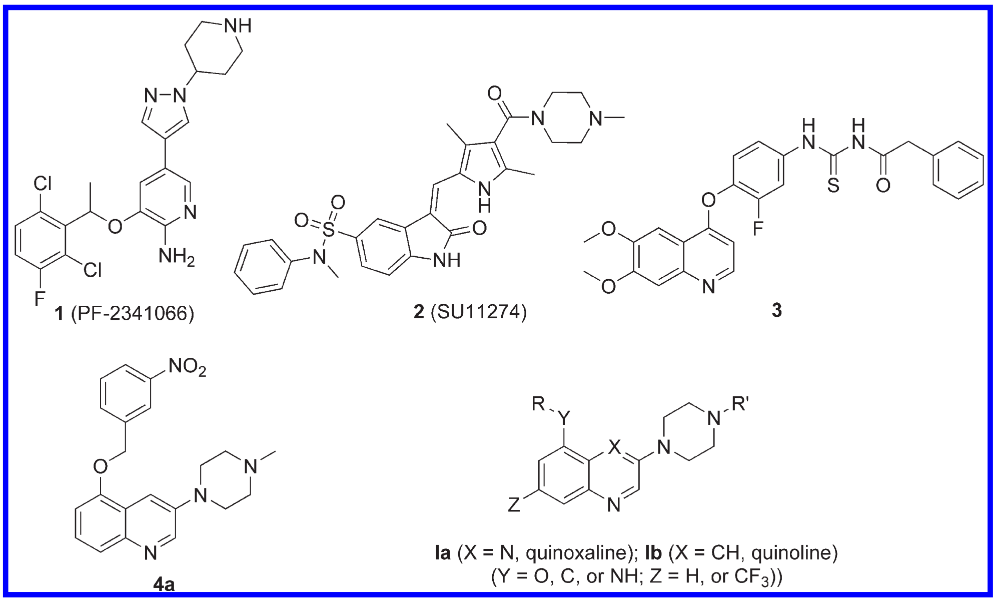
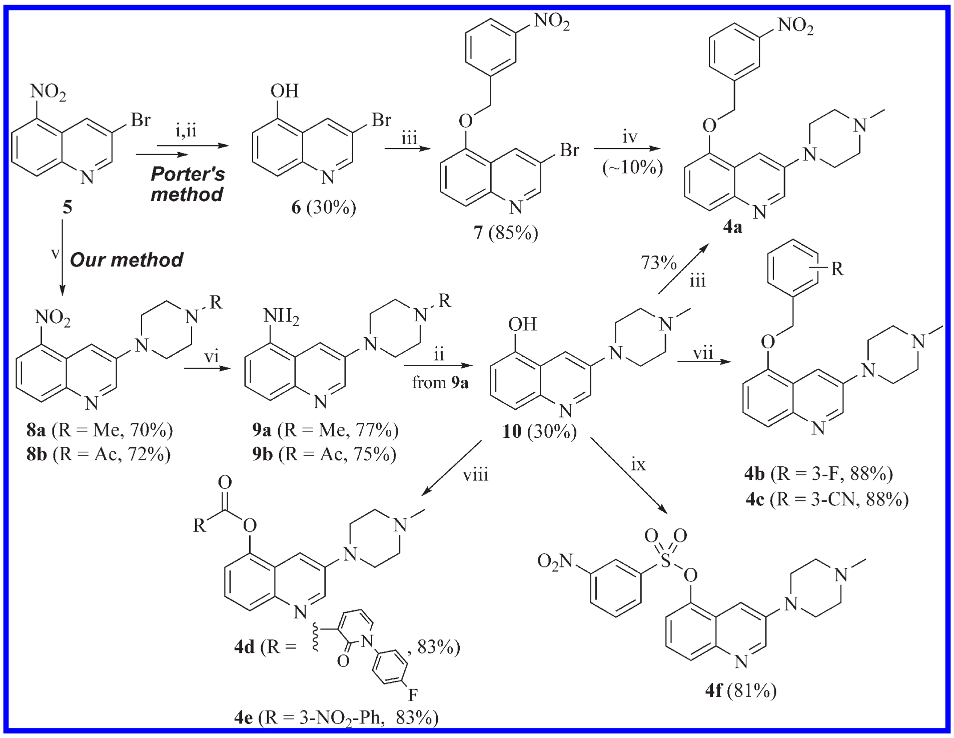


Figure 1. Representative c-Met inhibitors (**1**−**4a**) and multisubstituted quinoxalines or quinolines (**Ia**, **Ib**).

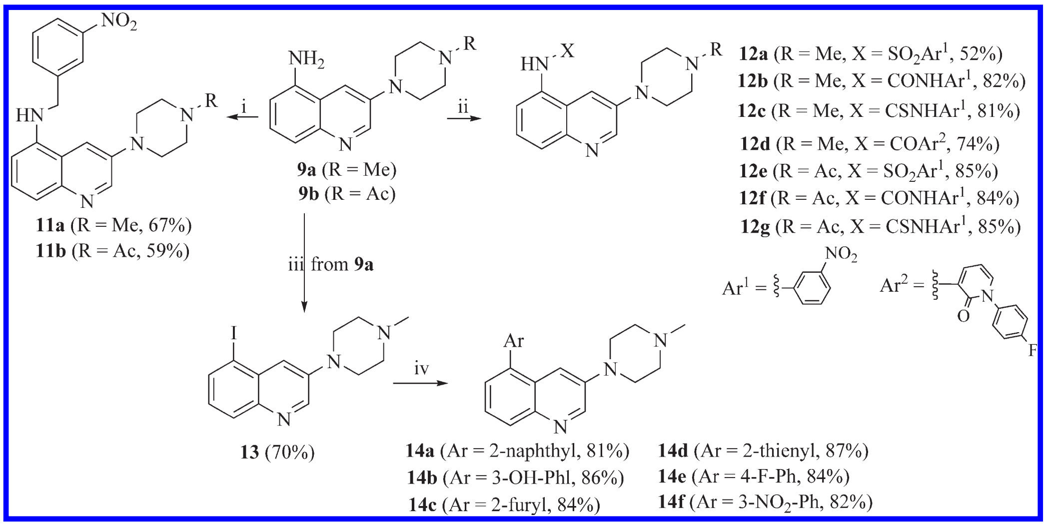
Scheme 1. Synthesis of Compounds 4a−f[a]



[a] Reagents and conditions: (i) Fe, AcOH, reflux; (ii) $NaNO_2$, AcOH, water, H_2SO_4, 0°C; then 10% aq H_2SO_4, reflux; (iii) 3-nitrobenzyl bromide, Cs_2CO_3, THF, 25 °C; (iv) $Pd_2(dba)_3$, BINAP, NaO^{t}Bu, *N*-methylpiperazine, toluene, reflux; (v) $Pd_2(dba)_3$, BINAP, Cs_2CO_3, *N*-methylpiperazine or *N*-acetylpiperazine, toluene, reflux; (vi) Fe, NH_4Cl, EtOH; (vii) 3-fluorobenzyl bromide (for **4b**) or 3-(bromomethyl)benzonitrile (for **4c**), Cs_2CO_3, THF, 25 °C; (viii) TBTU, DIPEA, 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid or 3-nitrobenzoic acid; (ix) 3-nitrobenzene-1-sulfonyl chloride, Et_3N, CH_2Cl_2.

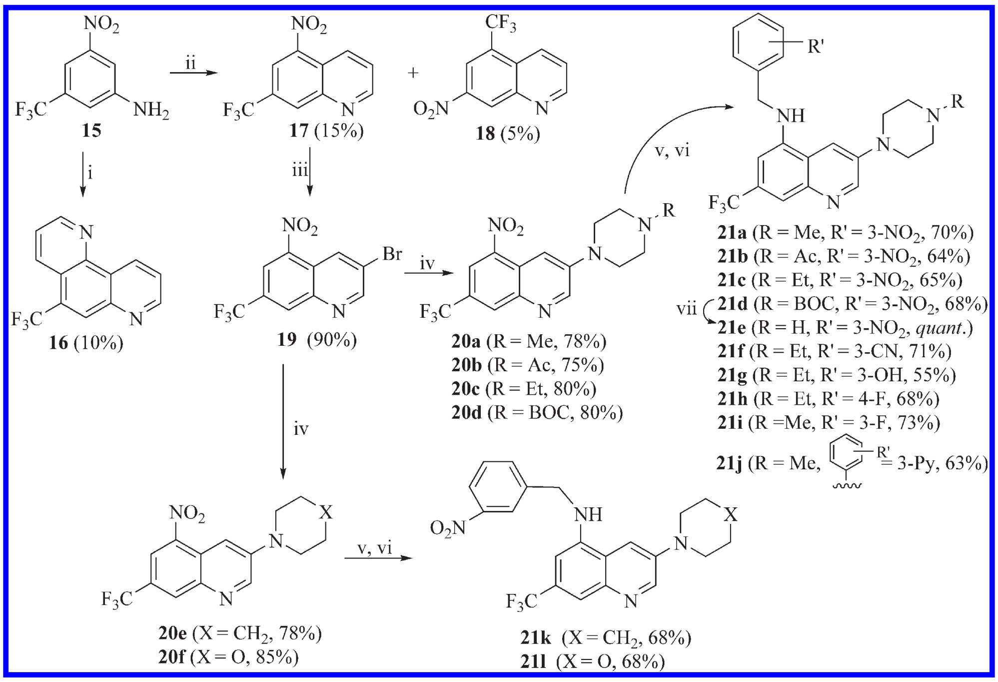
quinolines (**Ib**), only one single compound (**4a**) was reported, therefore leaving this chemotype largely unexplored. As shown in Scheme 1, the major obstacle in preparing compounds like **4a** using Porter's procedure is the low efficiency in introducing the quinolyl 3-substituent through a Buchwald−Hartwig C−N coupling reaction between bromide 7 and corresponding substituted piperazine (less than 10% yield).[25] We envisioned that the low yield in the C−N coupling reaction stems from the overall low reactivity of the quinoline system as well as the steric and electron-donating properties of the C5-substituent in precursor 7. During our natural-products-inspired drug discovery program,[26,27] we recently found that such difficulty in preparing

Scheme 2. Synthesis of Compounds 11a,b, 12a−g, and 14a−f[a]



[a] Reagents and conditions: (i) 3-nitrobenzaldehyde, EtOH, reflux, then $NaBH_4$, EtOH; (ii) 1-isothiocyanato-3-nitrobenzene or 1-isocyan-ato-3-nitrobenzene, TBTU, DIPEA, CH_2Cl_2, or 3- nitrobenzene-1-sulfonyl chloride, Et_3N, or 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid, TBTU, DIPEA, CH_2Cl_2; (iii) HBF_4, isoamyl nitrite, acetone, −8°C, then KI; (iv) arylboronic acid, $PdCl_2$(dppf), K_3PO_4, THF.

Scheme 3. Synthesis of Compounds 21a−l[a]



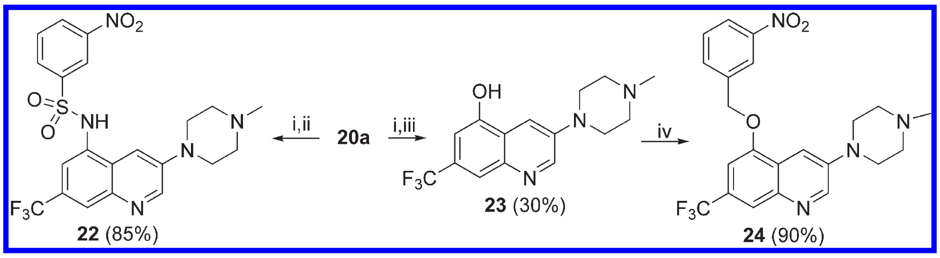
[a] Reagents and conditions: (i) conc H_2SO_4, glycercol, sodium 3-nitrobenzenesulfinate; (ii) conc H_2SO_4, glycercol, As_2O_5; (iii) NBS, AcOH; (iv) $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene, reflux; (v) Fe, NH_4CL, EtOH/H_2O, for **20a−c**; Pd/C, EtOAc, for **20d**; (vi) 3-nitrobenzaldehyde or 3-formylbenzonitrile or 3-hydroxybenzaldehyde or 4-fluorobenzaldehyde, EtOH, reflux; then $NaBH_4$, EtOH; (vii) TFA, CH_2Cl_2.

compound **4a** could be overcome by introducing the C3-amino substituent prior to the installation of the quinolyl-5-substituent. By use of this strategy, a series of 3,5-disubstituted and 3,5,7-trisubstituted quinolines were readily prepared in good yields, facilitating the discovery of a series of high potent c-Met inhibitors. In this report we disclosed the synthesis and c-Met kinase activity of these new quinoline derivatives.

CHEMISTRY

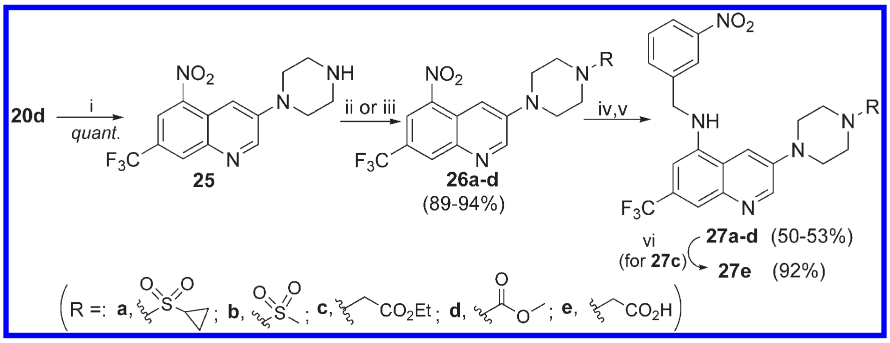
The synthesis was started from 3-bromo-5-nitroquinoline (**5**)[25] as the key intermediate (Scheme 1). First, we followed the procedure reported by Porter[25] to prepare 3-(4-methylpiperazin-1-yl)-5-(3-nitrobenzyloxy)quinoline (**4a**). Reduction of quinoline **5** with Fe/HOAc followed by diazotization and acidic hydrolysis (Sandmeyer process[28]) provided

Scheme 4. Synthesis of Compounds 22 and 24[a]



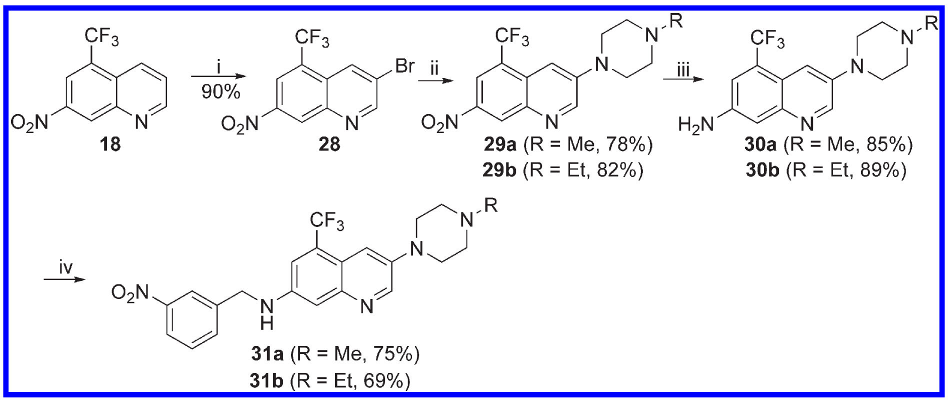
[a] Reagents and conditions: (i) Fe, NH_4Cl, $EtOH/H_2O$, reflux; (ii) 3-nitrobenzene-1-sulfonyl chloride, Et_3N,CH_2Cl_2; (iii) $NaNO_2$, AcOH, H_2O, H_2SO_4, 0°C; then 10% aq H_2SO_4, reflux; (iv) 3-nitrobenzyl bromide, Cs_2CO_3, THF, 25 °C.

Scheme 5. Synthesis of 27a–d[a]



[a] Reagents and conditions: (i) TFA, CH_2Cl_2; (ii) Et_3N, CH_2Cl_2, cyclopropanesulfonyl chloride or methanesulfonyl chloride or methyl carbonochloridate; (iii) ethyl 2-bromoacetate, K_2CO_3, accetone; (iv) Fe, NH_4Cl, $EtOH/H_2O$; (v) 3-nitrobenzaldehyde, EtOH,reflux; then $NaBH_4$, EtOH; (vi) $LiOH \cdot H_2O$, $THF/MeOH/H_2O$.

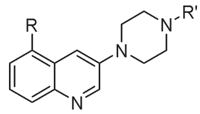
Scheme 6. Synthesis of Quinolines 31a,b[a]

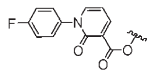
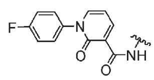


[a] Reagents and conditions: (i) NBS, AcOH, 120°C; (ii) $Pd_2(dba)_3$, BINAP, Cs_2CO_3, *N*-methylpiperazine or *N*-ethylpiperazine, toluene; (iii) Fe, NH_4Cl, $EtOH/H_2O$; (iv) 3-nitrobenzaldehyde, EtOH, reflux; then $NaBH_4$, EtOH.

3-bromo-5-hydroxyquinoline[25] (**6**) in 30% overall yield. Alkylation of quinoline **6** with 3-nitrobenzylbromide yielded ether **7**[25] in 85% yield. The subsequent Buchwald–Hartwig C–N coupling[29] ($Pd_2(dba)_3$, BINAP, NaO^{t}Bu) of bromide **7** with *N*-methylpiperazine proved to be very sluggish and afforded the desired product **4a** in miserable yield (5–10%) even after reflux for 2 days. Increasing the catalyst loading or shifting to other catalytic systems ($Pd(PPh_3)_4$/BINAP, $Pd(PPh_3)_4$/(*o*-tolyl)$_3$P, $Pd_2(dba)_3$/dppf)[29] or using other bases (Cs_2CO_3, K_3PO_4, KO^{t}Bu) did not lead to significant enhancement. Instead the de-bromo product was accumulated. As mentioned earlier, the poor efficiency of the coupling reaction is likely due to the steric and the electron-donating (more likely) effects of the C5-substituent in quinoline **7**. In this regard, we decided to conduct the C–N coupling reaction prior to the Sandmeyer reaction. To our delight, the coupling reaction between 5-nitro-3-bromoquinoline **5** and N-substituted piperazines under $Pd_2(dba)_3$/BINAP/Cs_2CO_3 catalytic system[30] proceeded smoothly and provided *N*-methyl and *N*-acetyl products **8a** and **8b** in 70% and 72% yields, respectively. Reduction[30] of **8a** and **8b** with

Table 1. c-Met Enzymatic Activity of 3,5-Disubstituted Quinolines[a]

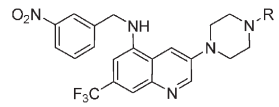
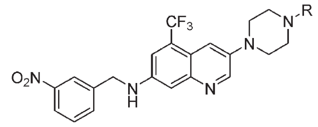


Compound	R	R'	IC_{50} (μM)
2	-	-	0.014(0.02[b])
4a	$3\text{-}NO_2\text{-}Ph\text{-}CH_2O\text{-}$	Me	0.14(0.057[c])
4b	$3\text{-}F\text{-}Ph\text{-}CH_2O\text{-}$	Me	25.8%@10μM
4c	$3\text{-}CN\text{-}Ph\text{-}CH_2O\text{-}$	Me	12.3±2.1
4d		Me	12.6%@10μM
4e	$3\text{-}NO_2\text{-}Ph\text{-}CO_2\text{-}$	Me	7.3%@10μM
4f	$3\text{-}NO_2\text{-}Ph\text{-}SO_3\text{-}$	Me	1.2±0.26
11a	$3\text{-}NO_2\text{-}PhCH_2\text{-}NH\text{-}$	Me	0.166±0.02
11b	$3\text{-}NO_2\text{-}PhCH_2\text{-}NH\text{-}$	Ac	0.11±0.03
12a	$3\text{-}NO_2\text{-}Ph\text{-}SO_2\text{-}NH$	Me	3.44±1.85
12b	$3\text{-}NO_2\text{-}Ph\text{-}NH\text{-}(C{=}O)\text{-}NH\text{-}$	Me	25.2%@10μM
12c	$3\text{-}NO_2\text{-}Ph\text{-}NH\text{-}(C{=}S)\text{-}NH\text{-}$	Me	12.4%@10μM
12d		Me	5.3±2.5
12e	$3\text{-}NO_2\text{-}Ph\text{-}SO_2\text{-}NH\text{-}$	Ac	2.56±0.26
12f	$3\text{-}NO_2\text{-}Ph\text{-}NH\text{-}(C{=}O)\text{-}NH\text{-}$	Ac	22.4±17.3
12g	$3\text{-}NO_2\text{-}Ph\text{-}NH\text{-}(C{=}S)\text{-}NH\text{-}$	Ac	13.9%@10μM
14a	2-Naphthyl	Me	5.80±1.90
14b	3-OH-Ph-	Me	30.7%@10μM
14c	2-Furyl	Me	12.7±2.4
14d	2-Thienyl	Me	14.3±4.4
14e	4-F-Ph-	Me	29.9%@10μM
14f	$3\text{-}NO_2\text{-}Ph\text{-}$	Me	2.3±0.9

[a] In vitro kinase assays were performed with the indicated purified recombinant c-Met kinase domains. [b] Data from ref 23. [c] Data from ref 25. IC_{50} values were calculated by the Logit method from the results of at least three independent tests with six concentrations each and expressed as the mean ± SD.

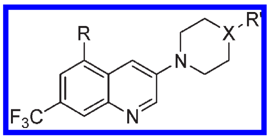
Fe/NH_4Cl gave aminoquinolines **9a** and **9b** in moderate yields. With success in introducing the desired 3-substituent, the subsequent diazotization and acidic hydrolysis occurred readily by following Porter's procedures, thus converting 5-aminoquinoline **9a** to 5-hydroxyquinoline **10** in 30% overall yield. Alkylation of **10** with 3-nitrobenzyl bromide provided target compound **4a** in 73% yield. Therefore, by switching of the reaction sequences, Porter's initial hit **4a** was practically prepared in four steps from 3-bromo-5-nitroquinoline (**5**). Following this improved process, alkylation of **10** with other bromides afforded 3,5-disubstituted

Table 2. c-Met Enzymatic Activity of 3,5,7-Trisubstituted Quinolines[a]

Compound	R	IC_{50} (μM)
		
21a	Me	0.00093±0.00015
21c	Et	0.0010±0.0005
		
31a	Me	8.9% @ 10μM
31b	Et	46.6% @ 10μM

[a] IC_{50} values were calculated by the Logit method from the results of at least three independent tests with six concentrations each and expressed as the mean ± SD.

Table 3. c-Met Enzymatic Activity of 3,5,7-Trisubstituted Quinolines[a]



compd	R	X-R′	IC_{50} (nM)
21a	3-NO_2-Ph-CH_2-NH-	N-Me	0.93 ± 0.15
21b	3-NO_2-Ph-CH_2-NH-	N-Ac	0.95 ± 0.13
21c	3-NO_2-Ph-CH_2-NH-	N-Et	1.01 ± 0.54
21d	3-NO_2-Ph-CH_2-NH-	N-BOC	10.6 ± 1.2
21e	3-NO_2-Ph-CH_2-NH-	N-H	2.2 ± 1.2
21f	3-CN-Ph-CH_2-NH-	N-Et	5.6 ± 0.17
21g	3-OH-Ph-CH_2-NH-	N-Et	230 ± 11
21h	4-F-Ph-CH_2-NH-	N-Et	10 000
21i	3-F-Ph-CH_2-NH-	N-Me	450 ± 49
21j	3-Py-CH_2-NH-	N-Me	160 ± 28
21k	3-NO_2-Ph-CH_2-NH-	CH_2	3.4 ± 0.68
21l	3-NO_2-Ph-CH_2-NH-	O	0.72 ± 0.17
22	3-NO_2-Ph-SO_2-NH-	N-Me	99 ± 45
24	3-NO_2-Ph-CH_2-O-	N-Me	2.7 ± 2.4
27a	3-NO_2-Ph-CH_2-NH-	N-SO_2-Pr-*c*	0.84 ± 0.32
27b	3-NO_2-Ph-CH_2-NH-	N-SO_2-Me	0.82 ± 0.06
27c	3-NO_2-Ph-CH_2-NH-	N-CH_2-CO_2Et	0.77 ± 0.11
27d	3-NO_2-Ph-CH_2-NH-	N-CO_2Me	43 ± 26
27e	3-NO_2-Ph-CH_2-NH-	N-CH_2-CO_2H	4.4 ± 1.9

[a] IC_{50} values were calculated by the Logit method from the results of at least three independent tests with six concentrations each and expressed as the mean ± SD.

quinolines **4b,c** in 88% yield (Scheme 1). Condensation[31] of 5-hydroxyquinoline **10** with 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid or 3-nitrobenzoic acid provided corresponding esters **4d** and **4e** in 83% yield. Esterification[32] of 5-hydroxyquinoline **10** with 3-nitrobenzene-1-sulfonyl chloride yielded 3,5-disubstituted quinoline **4f** in 81% yield.

Reductive[33,34] amination of 5-aminquinolines **9a** and **9b** with 3-nitrobenzaldehyde yielded corresponding 3,5-diaminoquinolines **11a** and **11b** in 67% and 59% yields, respectively (Scheme 2). Treating 5-aminoquinoline **9a** or **9b** with an appropriate isocyanate or isothiocyanate[35] provided compounds **12a**–**g** in moderate to good yields (52–85%). Diazotization[36] of 5-aminoquinoline **9a** followed by treatment with KI provided iodide **13** in 70% overall yield. Suzuki coupling[37] of **13** with an appropriate arylboronic acid yielded 5-aryl-3-aminoquinolines **14a**–**f** in 81–87% yields.

Since Porter[25] has reported that introducing a CF_3 substituent in quinoxaline analogues (**Ia**) led to an enhancement in c-Met enzymatic potency, we then explored if this can be transferred to the quinoline scaffold. Therefore, a series of 3,5,7-trisubstituted quinolines bearing a CF_3 group at the C7 position were prepared. As described in Scheme 3, following a procedure reported by Belcher[38] in 1954, 5-nitro-7-(trifluoromethyl)quinoline (**17**) was prepared by treating the commercially available 3-nitro-5-(trifluoromethyl)aniline (**15**) with glycerol, sulfuric acid, and arsenic pentoxide in low yield (15%), together with isolation of a regioisomer **18** as a side product (5%). The low yield in this cyclization reaction is primarily ascribed to the harsh reaction conditions and the difficulty in isolation of the products from the massive gummy reaction complex. Efforts to replace the toxic arsenic pentoxide by using other oxidants that were generally used in the Skraup reaction[39–42] (such as sodium 3-nitrobenzenesulfinate or green vitriol) were not successful. Instead, the double-cyclization product 5-(trifluoromethyl)-1,7-phenanthroline (**16**)[43] was isolated as the major product.

Similarly, treating quinoline **17** with NBS in AcOH provided bromide **19** in 90% yield (Scheme 3). The C–N coupling of quinoline **19** with appropriate 4-substituted piperazines afforded 5-nitro-7-trifluoromethylquinolines **20a**–**d** in 75–80% yields. Treating compounds **20a**–**c** with Fe/NH_4Cl followed by reductive amination[33,34] with appropriate aryl aldehydes and $NaBH_4$ yielded corresponding 3,5,7-trisubstituted quinolines **21a**–**j** in 55–73% overall yields. Coupling of bromide **19** with piperidine and morpholine provided **20e** and **20f** in 75% and 85% yields, which were then subjected to Fe-mediated reduction followed by reductive amination to yield quinolines **21k** and **21l** in 68% yields. In addition, treating 5-nitroquinoline **20a** with Fe/NH_4Cl followed by condensation with 3-nitrobenzenesulfonyl chloride provided sulfonic amide **22** in 85% yield (Scheme 4). Similarly, reduction and then Sandmeyer reaction[28] of 5-aminquinolines **20a** led to 5-hydroxyquinoline **23** in 30% overall yield. Alkylation of **23** with 3-nitrobenzyl bromide yielded ether **24** in 90% yield.

Further, compounds with variant substituents on the N-4 of piperazinyl moiety were also prepared (Scheme 5). Deprotection of **20d**, followed by treatment with an appropriate chloride or bromide provided quinolines **26a**–**d** in 89–94% yields. Reduction of the nitro group followed by reductive amination yielded 3,5,7-trisubstituted quinolines **27a**–**d** in 50–53% overall yields. Acid **27e** was formed in 92% yield by hydrolysis of ester **27c** with LiOH.

Meanwhile, the regioisomer **18**, the side product formed in the cyclization of aniline **15**, was also converted to the corresponding trisubstituted quinolines (Scheme 6). Therefore, treating quinoline **18** with NBS yielded bromide **28** in 90% yield. C–N coupling of bromide **28** with *N*-Me or *N*-Et substituted piperazines went

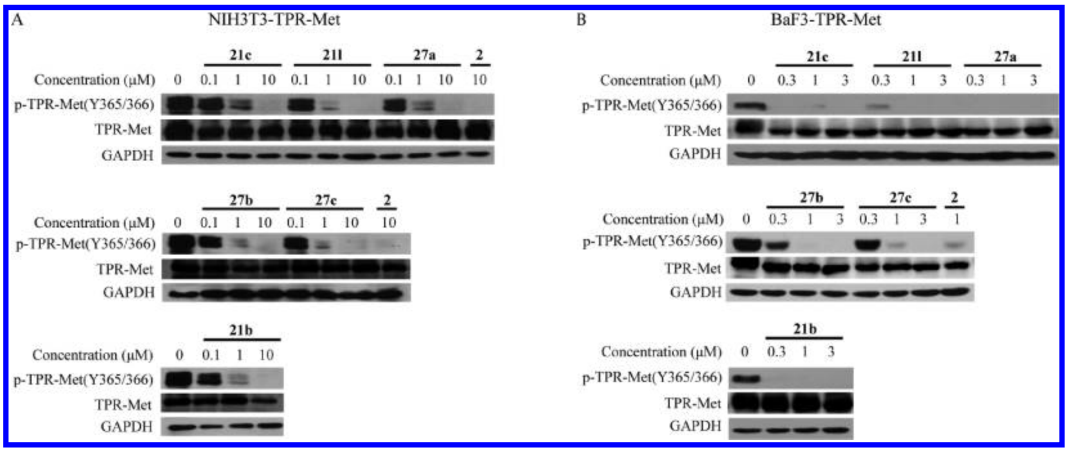


Figure 2. Effects of the selected quinoline compounds on c-Met phosphorylation in NIH3T3-TPR-Met and BaF3-TPR-Met cells.

Table 4. Effects of Selected Quinoline Compounds on Cell Proliferation[a]

	IC_{50} (μM)			
compd	BaF3-TPR-Met/-IL3	BaF3-TPR-Met/+IL3	NIH3T3-TPR-Met	NIH3T3
21b	0.39 ± 0.06	12.2 ± 2.0	2.0 ± 0.13	≥50
21c	0.37 ± 0.22	10.1 ± 3.8	2.6 ± 1.0	13.2 ± 5.2
21l	4.2 ± 3.3	≥50	4.0 ± 1.2	≥50
27a	0.50 ± 0.14	≥50	1.4 ± 0.16	≥50
27b	8.8 ± 2.6	≥50	2.2 ± 1.2	≥50
27c	1.4 ± 0.15	14.1 ± 5.0	5.9 ± 1.7	≥50
2	0.53 ± 0.14	6.4 ± 0.78	6.5 ± 0.42	≥50

[a] IC_{50} values are shown as the mean ± SD (μM) from three separate experiments.

through smoothly and provided 3,5,7-trisubstituted quinolines **29a,b** in 78% and 82% yields, respectively. Reduction of **29a,b** with Fe/NH_4Cl following by reductive amination yielded the target compounds **31a,b** in moderate yields.

■ RESULTS AND DISCUSSION

Enzymatic Assay and Lead Generation. All the newly synthesized 3,5-disubstituted quinolines, including C5 O-substituted (**4a**−**f**), C5 N-substituted (**11a**, **11b**, **12a**−**g**), and C5 aryl-substituted (**14a**−**f**) 3-aminoquinolines, and 3,5,7-trisubstituted quinolines **21a**−**l**, **22**, **24**, **27a**−**e**, and **31a,b** were evaluated for their ability to inhibit enzymatic activity of the c-Met receptor using a similar procedure reported in the literature.[44−48] Compound **4a** has been reported previously by Porter[25] (IC_{50} = 57 nM), and re-evaluated in our assay, showing an IC_{50} of 140 nM. The clinical compound **2**, one of the earliest c-Met inhibitors that was well characterized both in the literature and in our lab, was used as the reference compound, although more potent inhibitors have been reported[15−21] recently. The results are summarized in Tables 1−3.

First, a series of quinolines with diversified 3,5-disubstituents were evaluated as direct analogues of hit **4a**[25] (Table 1). Among them, compounds **4a**−**f** represented a subseries of compounds bearing an O-linkage on the C5 position of quinoline core. Surprisingly, ethers **4b** and **4c** displayed low enzymatic inhibition at 10 μM. Even less potency was observed on the 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic (**4d**) and benzoic (**4e**) quinolines. It was intriguing, however, that 3-nitrobenzenesulfonyl ester **4f** displayed moderate c-Met inhibition with an IC_{50} of 1.2 μM, although 9-fold less potent than hit **4a**. These results indicated that the C5 O-linkage does not bring good c-Met potency except compound **4a**. Significant differences were observed among quinolines **11a**, **11b**, and **12a**−**g** which contain an N-linkage on the C5 position. 3-Nitrobenzylamino substituted quinoline **11a** displayed high inhibitory potency with an IC_{50} of 0.166 μM, compatible with that of ether **4a**. Replacing the *N*-Me group in the piperazinyl fragment with an acyl group led to compound **11b** possessing a slightly enhanced potency (IC_{50} = 0.11 μM). These results indicated that the C5 N-linkage is beneficial to the ligand/receptor interaction, similar to Porter's results[25] on quinoxaline series (**Ia**). Sulfonylamides **12a** and **12e** displayed remarkably reduced potency with IC_{50} of 2−4 μM. Complete loss of c-Met inhibition was observed from compounds **12b**, **12c**, **12f**, and **12g** bearing a C5-ureido or thioureido substituent. Arylamido analogue **12d** retained somewhat inhibitory potency (IC_{50} = 5.3 μM). Compounds **14a**−**f** represented another subfamily of 3,5-disubstituted quinoline analogues bearing a C-linkage on the C5 position. Unfortunately, all these compounds displayed very low inhibitory effects on enzyme expressing the c-Met receptor (Table 1), similar to the results of the quinoxaline series reported by Porter.[25]

On the basis of the results above, 3-nitrobenzyloxy (**4a**) and 3-nitrobenzylamino (**11a,b**) groups turned out to be the optimal C5 functionality in the 3,5-disubstituted quinoline series, leading

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Table 5. In Vitro Pharmacokinetic Profile of c-Met Selective Compounds

	iv (10 mg/kg)[a]			po (20 mg/kg)[a]			
compd	CL ((L/h)/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	F (%)
21b	3.59 ± 0.23	16.7 ± 1.2	3.25 ± 0.68	316 ± 38	3.00 ± 0.0	2096 ± 483	37.5
27b	2.60 ± 0.36[b]	1.72 ± 0.73[b]	0.97 ± 0.04[b]	59.3 ± 26.0	1.33 ± 0.58	366 ± 113	3.7
27c	2.43 ± 0.60	1.36 ± 0.12	1.13 ± 0.56	279 ± 66	0.50 ± 0.43	700 ± 147	8.2

[a] Values are the average of three runs. Vehicle: DMSO, Tween 80, normal saline. CL, clearance; V_{ss}, volume of distribution; $T_{1/2}$, half-life; C_{max}, maximum concentration; T_{max}, time of maximum concentration; $AUC_{0-\infty}$, area under the plasma concentration time curve; F, oral bioavailability. [b] Dose was reduced to 2.5 mg/kg because of poor solubility.

to high c-Met potency. These two functionalities were therefore transferred to the 3,5,7-trisubstituted quinoline series.

As shown in Table 2, compounds **21a**, **21c**, **31a**, and **31b** were first evaluated to determine the effects of substitution regiochemistry on c-Met inhibitory potency. To our delight, incorporation of a C7-CF_3 substituent to quinolines **11a** led to 3,5,7-trisubstituted quinoline **21a**, exhibiting extraordinarily elevated potency with an IC_{50} of 0.93 nM, 58-fold more potent than non-CF_3-containing compound **11a**. Similar high potency (IC_{50} = 1.01 nM) was observed on compound **21c** bearing an *N*-Et group on the piperazinyl fragment. However, switch of the C5- and C7-substituents in compounds **21a** and **21c** yielded the corresponding 3,5,7-trisubstituted quinolines **31a** and **31b**, showing nearly complete loss of c-Met inhibitory potency. This result clearly indicated that both the CF_3-substituent and the regiochemistry of C5- and C7-substitution patterns are of significant importance to the enzymatic inhibition potency. Although the crystal structure of our compounds with c-Met are not available, an analogous interaction model can be drawn on the basis of the X-ray crystal structure of c-Met with Porter's quinoxalines,[25] where the N-1 atom of the current quinoline compounds may form a H-bond to Met 1160 in the hinge region of the kinase while the C7-CF_3 and C5-aryl groups would occupy the hydrophobic pockets and the piperazine function should be positioned toward solvent. Therefore, a broad range of 3,5,7-trisubstituted quinoline analogues of **21a** bearing a C7-CF_3 function but with diversified C3- and C5-substituents were further designed (Table 3).

First, with 3-nitrobenzylamino group as the "solid" C5-substituent, various N-substituted piperazinyl side chains (**21a**−**e**) were explored as the C3-substituent in the 3,5,7-trisubstituted quinoline core. It was found that all substituents either with electron-donating property, such as *N*-Me (**21a**) and *N*-Et (**21c**), or with electron-withdrawing nature, such as *N*-Ac (**21b**), offered similar high potency with IC_{50} of around 1.0 nM. The bulky effect of the substituent likely had some effect on the potency, since the more steric analogue **21d** showed 5-fold lower potency (10.6 nM) than the nonsubstituted analogue **21e** (2.2 nM). Next, with *N*-Me or *N*-Et as the optimal piperazin-4-substituents, a series of 3,5,7-trisubstituted quinolines with diversified C5-substituents were explored. Slightly lower potency (5.5-fold) was observed on 3-CN-Ph substituted analogue **21f**. Analogue **21g** with an electron-donating substituent (3-OH-Ph) gave much lower potency than **21f** and is 227-fold less potent than 3-NO_2-Ph substituted parent **21c**, indicating that an additional H-bonding donor did not contribute positively to the c-Met activity. Both 4-F-Ph and 3-F-Ph substituted analogues **21h** and **21i** displayed remarkably reduced potency, but the 3-substituted analogue **21i** is much more potent than the 4-substituted version **21h** with IC_{50} of 10 and 0.45 μM, respectively. Moderate potency (0.16 μM) was observed on pyridine-3-yl analogue **21j**. Sulfonylamide **22** gave reasonable potency (99 nM), whereas 3-NO_2 substituted benzyloxy compound **24** presented good potency of 2.7 nM.

Table 6. Kinase Selectivity Profile of Compound 21b

kinase	enzyme IC_{50} (nM)	kinase	enzyme IC_{50} (nM)
RON	>10 000	ErbB2	>10 000
Flt-1	>10 000	c-Src	>10 000
KDR	>10 000	Abl	>10 000
c-Kit	>10 000	EPH-A2	>10 000
PDGFRα	>10 000	EPH-B2	>10 000
RET	>10 000	IGF1R	>10 000
EGFR	>10 000	FGFR1	>10 000

From the results above, the 3-nitrobenzylamino group remains as the best C5 function in the 3,5,7-trisubstitued quinoline scaffold. Therefore, with this substitution pattern fixed, we further exploited the nature and limitation of the substituent on the *N*-4 position of the piperazine component. This site is also a potential position to improve the aqueous solubility.[25] Notably, compounds **27a**−**c** and **21l** displayed exceptionally high potency with IC_{50} in the subnanomolar range (0.72−0.84 nM). However, compound **27d** bearing a one-carbon shorter *N*-alkyl ester side chain showed 56-fold lower potency (IC_{50} = 43.5 nM) than compound **27c**. Surprisingly, the corresponding acid **27e** displayed good potency of 4.4 nM. However, this compound suffered poor water solubility that limited its further development. It is of note that replacing a piperazine fragment with a piperidine functionality led to compound **21k**, also showing good potency with an IC_{50} of 3.4 nM. This result further confirms that the N-4 atom in the piperazine fragment is a solvent-interaction site and is not necessarily required for c-Met inhibition. Excellent potency was observed from compound **21l**, bearing a morpholine unit and showing an IC_{50} of 0.72 nM.

Effects on c-Met Phosphorylation. From the enzymatic results mentioned above, compounds **21a**−**c**, **21l**, and **27a**−**c** stood out as the most potent c-Met inhibitors among our synthetic quinolines with IC_{50} of less than 1.0 nM, much more potent than the reference compound **2** (IC_{50} = 14 nM). However, in view of the lower solubility observed in the preparation, compound **21a** was not carried ahead for further evaluation. To determine whether c-Met kinase inhibition of these compounds in cell-free system could be recapitulated in vitro, we extended our study to the two newly generated NIH-3T3 and BaF3 cell lines that stably express a constitutively active, ligand-independent, oncogenic form of c-Met (NIH-3T3-TPR-Met and BaF3-TPR-Met cells).[49,50] It was found that our newly synthetic

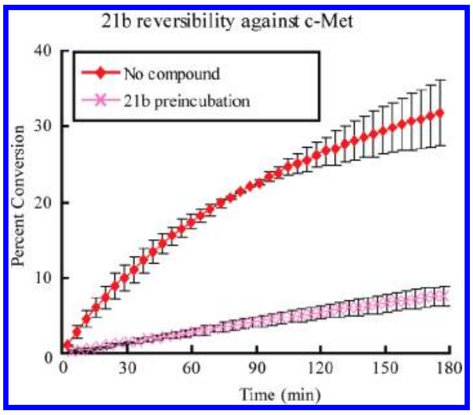

Figure 3. Compound **21b** reversibly binds to c-Met. Recombinant c-Met was preincubated with compound **21b** at a concentration of 100-fold IC_{50} for 30 min in the absence of the substrate and then diluted and assayed for enzyme activity. "Conversion" here represents the enzyme activity and means "fraction of peptide converted (phosphorylated) from substrate to product" [$C = P/(P + S)$].

c-Met inhibitors **21b**, **21c**, **21l**, and **27a**−**c** inhibited c-Met autophosphorylation in a dose-dependent manner in these two cell lines, similar to **2** (Figure 2).

Effects on Cell Proliferation. Since activation of c-Met ultimately results in cell proliferation, the inhibitory effects of these compounds on cell proliferation were then evaluated in both NIH-3T3-TPR-Met and BaF3-TPR-Met cells. As shown in Table 4, compounds **21b**, **21c**, **21l**, and **27a**−**c** markedly inhibited the proliferation of BaF3-TPR-Met cells. Compounds **21b**, **21c**, and **27a** showed much lower IC_{50} (0.23−0.50 μM), comparable to or even better than that of **2**. Similarly, these compounds also showed significant cell proliferation inhibition in the NIH-3T3-TPR-Met cells with IC_{50} of 1.4−6.5 μM, whereas lower potency was observed in non-TPR-Met expressed cells. In addition, compound **21c** demonstrated weak inhibition in the NIH3T3 cells, suggesting that there might be some other targets for **21c** in this cell. In general, compounds **21b**, **21c**, and **27a** have higher inhibitory effects than compounds **21l** and **27b**,**c** in the tested two cell lines. These results clearly demonstrated that Met is the main cellular target of growth inhibition of these compounds.

Pharmacokinetic (PK) Profiles of the Selected c-Met Inhibitors. Next, we assessed pharmacokinetic profiles of the highly potent compound **21b**. Compounds **27b** and **27c** were also evaluated as a comparison. As shown in Table 5, the PK parameters of compounds **21b**, **27b**, and **27c** were evaluated in mice after single iv (10 mg/kg) and po (20 mg/kg) administration. Different from their similar in vitro enzymatic potency, compounds **21b**, **27b**, and **27c** displayed remarkable differences in their pharmacokinetic properties. The acetyl substituted compound **21b** displayed a longer half-life (3.25 h), favorable clearance (3.59 (L/h)/kg, iv), and moderate oral bioavailability (37.5%), while *N*-methylsulfonyl and *N*-ethoxycarbonylmethyl analogues **27b** and **27c** showed much shorter half-lives (∼1 h) and poor bioavailability (less than 10%), probably because of their readily hydrolytic liability that leads to their low cellular permeability. Given the promising overall PK profile and structure novelty, compound **21b** was selected as the lead for subsequent in vitro and in vivo evaluation.

Compound 21b Is a Highly Selective c-Met Inhibitor. To examine whether lead compound **21b** is a selective c-Met inhibitor, it was screened against c-Met family member Ron along with other 12 tyrosine kinases, including Flt-1, KDR, c-Kit, PDGFRα, RET, EGFR, ErbB2, c-Src, Abl, EPH-A2, EPH-B2, IGF1R, and FGFR1. Compared to its high potency against c-Met (IC_{50} = 0.95 nM), compound **21b** produced more than 10000-fold less potency against these selected kinases, with IC_{50} greater than 10 μM (Table 6), indicating that compound **21b** is a highly specific c-Met-targeting inhibitor.

Next, we investigated whether compound **21b** reversibly binds to c-Met using a diluting method.[51] With this approach, 100 times normal reaction amount of enzyme was preincubated for 30 min with compound **21b** at a concentration that was 100-fold greater than its IC_{50}. DMSO functions as a vehicle control. After dilution of the enzyme/inhibitor mixture to 100-fold at the end of the incubation, the reaction buffer plus ATP and substrate were added and the enzyme activity was determined continuously. Generally, a reversible inhibitor would dissociate quickly allowing recovery of enzyme activity, whereas an irreversible inhibitor, in contrast, shall prevent recovery of enzyme activity. As shown in Figure 3, preincubation of c-Met with compound **21b** resulted in a gradual recovery of the enzyme activity, suggesting compound **21b** as a reversible c-Met inhibitor.

Compound 21b Inhibits c-Met Phosphorylation and Its Downstream Signaling Pathway. We next investigated the in vitro and in vivo kinase-targeting activity of **21b**. Both natural (MKN-45 human gastric carcinoma cells and H1993 human lung cancer cells that express elevated levels of constitutively active c-Met) and genetic (NIH-3T3-TPR-Met cells and BaF3-TPR-Met cells) c-Met expressing cell lines were selected. It was found that exposure to compound **21b** produced constitutive inhibition of c-Met phosphorylation in both MKN45 and H1993 cells, with a complete abolishment at 0.5 μM in H1993 cells (Figure 4A,B). In addition, Erk1/2 and AKT, the key downstream molecules of c-Met that play important roles in c-Met functioning,[2,52] were also significantly dephosphorylated upon compound **21b** treatment (Figure 4A,B). Similar results were recapitulated in NIH-3T3-TPR-Met and BaF3-TPR-Met cells (Figure 4C,D). These data supported that compound **21b** inhibits c-Met activity as well as subsequent c-Met downstream signaling.

Compound 21b Inhibits the Proliferation of c-Met-Addicted Human Cancer Cells. To determine the cytotoxic activity of compound **21b**, it was further evaluated on a panel of human cancer cell lines with different settings of c-Met expression/activation. As shown in Table 7, compound **21b** effectively inhibited the proliferation of human cancer cell lines; however, the IC_{50} varied widely among these cancer cells. Notably, compound **21b** showed IC_{50} of 1.0, 1.0, and 5.3 μM, respectively, in MKN45, SNU-5, and H1993 cell lines, which are the c-Met most sensitive cancer cell lines with c-Met both being amplified and overexpressed. In contrast, compound **21b** exerted much less antiproliferative effects with IC_{50} of around 50 μM in c-Met-less sensitive cell lines such as SNU-1, MDA-MB-231 NCI-H441, and BxPC3. These findings, together with the observation that compound **21b** significantly inhibited BaF3-TPR-Met and NIH-3T3-TPR-Met cell proliferation, strongly suggested that antiproliferative activity of compound **21b** against human cancer cell lines is dependent on the native status of the c-Met receptor.

Compound 21b Inhibits Tumor Growth in Vivo. To evaluate the inhibition of compound **21b** on c-Met activities in vivo, its antitumor activity was investigated in the NIH-3T3-TPR-Met xenograft model. In this model, tumor growth is specifically driven by c-Met activation that is independent of its endogenous

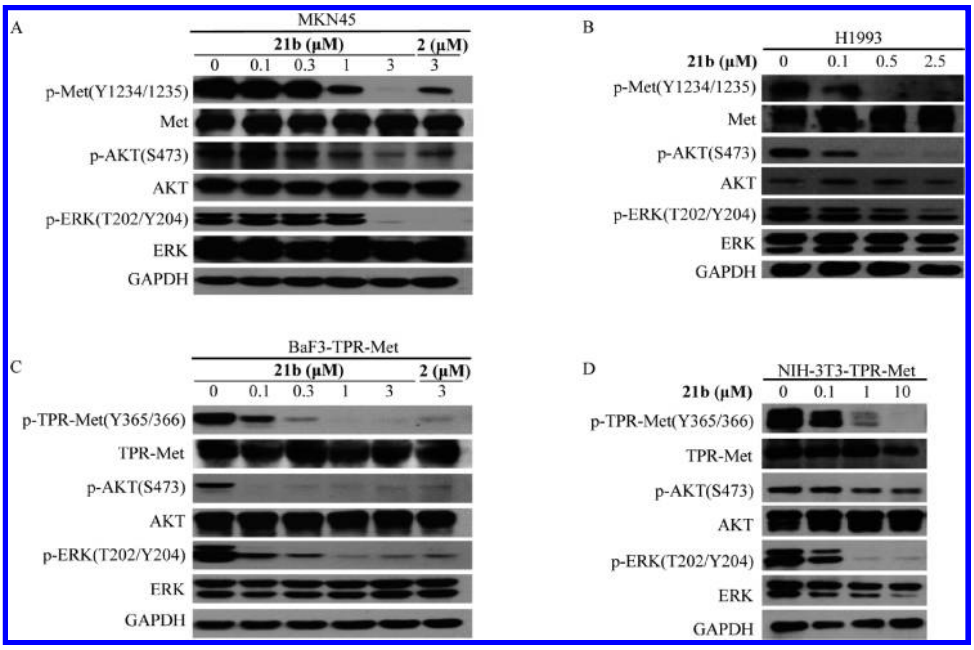

Figure 4. Dose-dependent inhibition of compound **21b** on c-Met phosphorylation and signal transduction pathways in MKN45 (A), H1993 (B), BaF3-TPR-Met (C), and NIH3T3-TPR-Met (D).

Table 7. Antiproliferative Activity of Compound 21b against Human Tumor Cell Lines[a]

	IC_{50} (μM)							
compd	MKN45	H1993	SNU-5	SNU-1	NCI-H441	MDA-MB-231	MCF-7	BxPC3
21b	1.0 ± 0.35	5.3 ± 2.10	1.0 ± 0.16	≥50	≥50	≥50	16 ± 3.6	46 ± 3.1
2	1.3 ± 0.58	7.3 ± 0.66	0.8 ± 0.16	7.0 ± 0.53	13 ± 2.8	11 ± 0.6	6.2 ± 0.82	16 ± 4.0

[a] IC_{50} values are shown as the mean ± SD (μM) from three separate experiments.

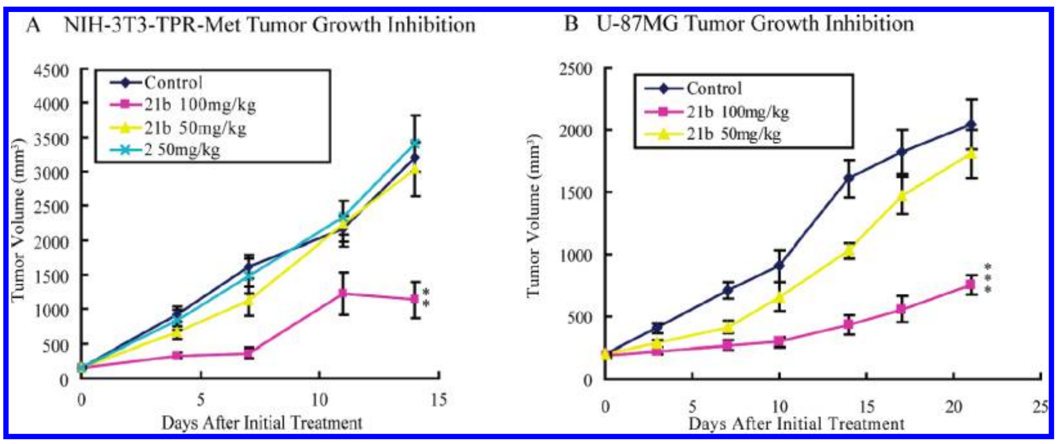

Figure 5. Antitumor efficacy of compound **21b** in the NIH3T3-TPR-Met (A) and U-87MG (B) xenograft models. Tumor-bearing nude mice were randomly divided into groups when tumor volume reached 100−200 mm^3 and given compound **21b** ip at indicated dose levels or vehicle alone over the designated treatment schedule. Data are presented as the mean ± SEM; $n = 6$ mice per group: (**) $P < 0.01$, (***) $P < 0.001$, versus control group, determined with Student's t test.

HGF ligand. We found that intraperitoneal (ip) injection of compound **21b** at doses of 100 mg/kg for 14 days significantly inhibited tumor growth of 68% ($p < 0.01$), compared with the vehicle control (Figure 5A). To further confirm the in vivo tumor growth arrest of compound **21b**, we extended the test to another well-accepted c-Met-dependent model, the U-87MG human globlastoma xenograft model. It was found that compound **21b**, at the same dosing regimens, significantly inhibited tumor growth up to 69% ($p < 0.001$) whereas a marginal effect on tumor growth was observed at doses of 50 mg/kg (Figure 5B).

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Meanwhile, compound **21b** was found to be well tolerated during the test and showed no significant loss of body weight in these two xenograft models (data not shown).

CONCLUSION

Following a reported c-Met inhibitor HTS hit **4a**, a comprehensive series (42) of 3,5-disubstituted and 3,5,7-trisubstituted quinolines were readily prepared by our improved synthetic strategy. Most of the 3,5-disubstituted quinolines displayed poor c-Met inhibitory activity, while introduction of a C7-trifluoromethyl group led to a novel class of 3,5,7-trisubstituted quinolines possessing extraordinary high c-Met inhibitory potency. Compound **21b** showed the most potent enzyme activity with IC_{50} of less than 1.0 nM and was selected as the lead for further evaluation. In addition to its promising overall PK profile, this compound inhibited c-Met autophosphorylation and markedly inhibited the proliferation in both NIH-3T3-TPR-Met and BaF3-TPR-Met cell models. Moreover, it inhibits both c-Met activation and the downstream signaling pathways. In the cytotoxic evaluation, compound **21b** effectively inhibited the proliferation of a panel of human cancer cell lines. At doses of 100 mg/kg, compound **21b** exhibited statistically significant tumor growth inhibition (68−69%) in both NIH-3T3-TPR-Met and U-87MG human gliobastoma xenograft models, and no obvious weight loss was observed.

Although many new more potent c-Met inhibitors have been reported recently, compound **21b** stood out as one of the most selective among the currently reported c-Met inhibitors. It displayed favorable in vitro and in vivo pharmacological profiles that warrant further investigation. More dosing regimens and overall safety evaluation are currently under investigation.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectral data were recorded in $CDCl_3$ on Varian Mercury 300 NMR spectrometer, and ^{13}C NMR was recorded in $CDCl_3$ on Varian Mercury 400 NMR spectrometer. Low-resolution mass spectra and high-resolution mass spectra were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Elemental analyses were performed on a CE 1106 elemental analyzer. Column chromatography was carried out on silica gel (200−300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Yields were of purified compounds and were not optimized. HPLC analysis was conducted for all compounds listed in Tables 1−3 on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; ZORBAX Eclipse XDB-C8, 4.8 mm × 150 mm, 5 μm, 1.0 mL/min, UV 254 nM, room temp) with two solvent systems ($MeCN/H_2O/TFA$, and $MeOH/H_2O/TFA$). All the assayed compounds displayed a purity of 95−99% in both solvent systems.

General Procedure for Preparation of 3-Substituted 5-Nitroquinolines 8a and 8b. A dried flask was charged with (±)-BINAP (16 mmol %), Cs_2CO_3 (1.4 mmol), 3-bromo-5-nitroquinoline **5** (1 mmol), $Pd_2(dba)_3$ (3.5 mmol %), *N*-methylpiperazine or *N*-acetylpiperazine (1.2 mmol), and anhydrous toluene (10 mL) under nitrogen. The mixture was heated to 110 °C and stirred for 8 h, then cooled to room temperature and filtered. The filtrate was concentrated in vacuum and purified by chromatography ($CHCl_3$/MeOH = 20:1) to yield corresponding quinolines **8a** and **8b**. For quinoline **8a**: yellow solid (70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.7 Hz, 1H), 8.30 (d, *J* = 8.1 Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 1H), 8.14 (d, *J* = 2.4 Hz, 1H), 7.45 (t, *J* = 8.1 Hz, 1H), 3.42 (t, *J* = 4.8 Hz, 4H), 2.60 (t, *J* = 4.8 Hz, 4H), 2.34 (s, 3H); MS (EI) *m/z* 272 (M^+). For quinoline **8b**: yellow solid (72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (d, *J* = 3.0 Hz, 1H), 8.36 (d, *J* = 6.6 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 2.7 Hz, 1H), 7.54 (t, *J* = 8.1 Hz, 1H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.72 (t, *J* = 5.1 Hz, 2H), 3.44 (m, 4H), 2.17 (s, 3H); MS (EI) *m/z* 300 (M^+).

General Procedure for Preparation of 3-Substituted 5-Aminoquinolines 9a and 9b. To a solution of quinoline **8a** or **8b** (1 mmol) in ethanol (10 mL) was added iron powder (7 mmol) followed by a solution of NH_4Cl (10 mmol) in water (4 mL). The mixture was heated at reflux until the starting material was completely consumed. Ammonia was added to quench the reaction, and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum, and purified by chromatography ($CHCl_3$/MeOH = 10:1) to afford the corresponding anilines **9a** and **9b**. For quinoline **9a**: yellow solid (77%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, *J* = 2.1 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.25 (m, 2H), 6.72 (d, *J* = 7.5 Hz, 1H), 4.12 (s, 2H), 3.22 (t, *J* = 4.8 Hz, 4H), 2.56 (t, *J* = 4.8 Hz, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5, 143.8, 143.4, 141.0, 126.6, 119.8, 119.1, 110.9, 110.7, 54.7, 49.1, 46.0; MS (EI) *m/z* 242 (M^+). For quinoline **9b**: yellow solid (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, *J* = 2.7 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.33 (m, 2H), 6.80 (d, *J* = 7.8 Hz, 1H), 4.08 (s, 2H), 3.84 (t, *J* = 5.1 Hz, 2H), 3.69 (t, *J* = 5.1 Hz, 2H), 3.28 (t, *J* = 5.1 Hz, 2H), 2.22 (t, *J* = 5.1 Hz, 2H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 144.8, 143.9, 143.3, 141.5, 127.2, 119.5, 118.8, 112.2, 110.6, 49.9, 49.1, 45.9, 40.9, 21.2; MS (EI) *m/z* 270 (M^+).

3-(4-Methylpiperazin-1-yl)-5-hydroxyquinoline (10). To a stirring solution of 5-aminoquinoline **9a** (968 mg, 4 mmol) in a mixture of $AcOH/H_2O/H_2SO_4$ (8:1:1 v/v/v, 4.5 mL) at 0 °C was added a solution of $NaNO_2$ (310 mg, 4.5 mmol) in water (0.53 mL). The reaction mixture was stirred at 0 °C for 30 min and then was added slowly to a boiling solution of 10% H_2SO_4 (30 mL). The mixture was heated at reflux for 5 min, then cooled, basified with ammonia to pH 8, and extracted with Et_2O (3 × 30 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuum. Purification by chromatography ($CHCl_3$/MeOH = 20:1) provided compound **10** (291.6 mg, 30%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (d, *J* = 2.7 Hz, 1H), 7.88 (d, *J* = 2.1 Hz, 1H), 7.56 (d, *J* = 2.1 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 3.32 (t, *J* = 4.5 Hz, 4H), 2.71 (t, *J* = 4.5 Hz, 4H), 2.45 (s, 3H); MS (EI) *m/z* 243 (M^+).

General Procedure for Preparation of Ethers 4a−c. To a solution of 5-hydroxyquinoline **10** (0.1 mmol) in THF (2 mL) at 0 °C was added Cs_2CO_3 (0.15 mmol). An appropriately substituted benzyl bromide (0.12 mmol) was added, and the resulting reaction mixture was stirred at room temperature for 2 h and then filtered. The filtrate was concentrated in vacuum and the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH = 30:1) to give the corresponding 3,5-disubstituted quinolines.

5-(3-Nitrobenzyloxy)-3-(4-methylpiperazin-1-yl)quinoline (4a)[25]. Brown solid (73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (d, *J* = 2.7 Hz, 1H), 8.44 (s, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 7.80 (m, 2H), 7.61 (m, 2H), 7.38 (t, *J* = 8.1 Hz, 1H), 6.85 (d, *J* = 7.5 Hz, 1H), 5.35 (s, 2H), 3.37 (t, *J* = 4.8 Hz, 4H), 2.66 (t, *J* = 4.8 Hz, 4H), 2.38 (s, 3H); MS (EI) *m/z* 378 (M^+).

5-(3-Fluorobenzyloxy)-3-(4-methylpiperazin-1-yl)quinoline (4b). Brown solid (88%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, *J* = 3.0 Hz, 1H), 7.79 (d, *J* = 3.0 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.36 (m, 2H), 7.22 (m, 2H), 7.02 (m, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 5.23 (s, 2H), 3.33 (t, *J* = 4.8 Hz, 4H), 2.63 (t, *J* = 4.8 Hz, 4H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 161.7, 152.9, 144.7, 144.3, 143.5, 139.4 (d, *J* = 7.2 Hz), 130.1 (d, *J* = 8.2 Hz), 125.8, 122.5, 121.6, 121.1, 114.8 (d, *J* = 21.1 Hz), 114.0 (d, *J* = 21.9 Hz), 111.6, 106.1, 69.5, 54.7, 49.0, 46.0; MS (EI) m/z 351 (M^+); HRMS calcd for $C_{21}H_{22}FN_3O$ (M^+) 351.1747, found 351.1755.

5-(3-Cyanobenzyloxy)-3-(4-methylpiperazin-1-yl)quinoline (4c). Brown solid (88%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (d, J = 3.0 Hz, 1H), 7.79 (s, 1H), 7.76 (d, J = 2.7 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.64 (m, 2H), 7.52 (t, J = 7.8 Hz, 1H), 7.36 (t, J = 8.1 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 5.27 (s, 2H), 3.34 (t, J = 5.1 Hz, 4H), 2.64 (t, J = 5.1 Hz, 4H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6, 144.9, 144.4, 143.6, 138.5, 131.7, 131.4, 130.6, 129.5, 125.7, 122.0, 121.0, 118.5, 112.8, 111.4, 106.2, 69.1, 54.7, 48.9, 46.0; MS (EI) m/z 358 (M^+); HRMS calcd for $C_{22}H_{22}N_4O$ (M^+) 358.1794, found 358.1789.

General Procedure for Preparation of Esters 4d and 4e. To a mixture of 5-hydroxyquinoline **10** (0.1 mmol), an appropriate carboxylic acid (0.11 mmol), and 2-(1*H*-benzotriazole-1-yl)-1,1,3-tetramethyluronium tetrafluoroborate (TBTU) (0.13 mmol) in DMF/CH_3CN (1:1, 4 mL) at 0 °C was added diisopropylethylamine (DIPEA, 0.36 mmol). The reaction mixture was stirred at room temperature for 2 h. Water (5 mL) was added to quench the reaction, and the resulting mixture was extracted with $CHCl_3$ (3 × 10 mL). The organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum, and purified by chromatography ($CHCl_3$/MeOH = 10:1) to yield the corresponding esters **4d** and **4e**.

3-(4-Methylpiperazin-1-yl)quinolin-5-yl-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine 3-Carboxylate (4d). Off-white solid (83%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (d, J = 2.7 Hz, 1H), 8.38 (dd, J = 2.4, 7.2 Hz, 1H), 7.93 (d, J = 2.7 Hz, 1H), 7.88 (d, J = 6.6 Hz, 1H), 7.66 (dd, J = 2.4, 7.2 Hz, 1H), 7.44 (m, 4H), 7.20 (m, 2H), 6.43 (t, J = 7.2 Hz, 1H), 3.33 (t, J = 5.1 Hz, 4H), 2.66 (t, J = 5.1 Hz, 4H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 159.1, 146.1, 145.4, 144.8 (2), 143.3, 143.1, 136.2, 128.4 (d, J = 8.8 Hz), 126.7, 125.0, 122.9, 121.8, 118.8, 116.3 (d, J = 23.0 Hz), 111.2, 105.0, 54.7, 48.6, 46.1; MS (EI) m/z 458 (M^+). Anal. ($C_{26}H_{23}FN_4O_3 \cdot 0.15H_2O$) C, H, N.

3-(4-Methylpiperazin-1-yl)quinolin-5-yl 3-Nitrobenzoate (4e). Off-white solid (83%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.83 (s, 1H), 8.60 (d, J = 6.6 Hz, 1H), 8.54 (d, J = 8.1 Hz, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 6.6 Hz, 1H), 7.27 (s, 1H), 3.29 (s, 4H), 2.58 (s, 4H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 148.4, 145.1, 144.9, 144.7, 143.3, 135.8, 130.9, 130.1, 128.2, 127.4, 125.1, 125.0, 122.5, 118.9, 109.1, 54.6, 48.5, 46.0; MS (EI) m/z 392 (M^+); HRMS calcd for $C_{21}H_{20}N_4O_4$ (M^+) 392.1485, found 392.1492.

3-(4-Methylpiperazin-1-yl)quinolin-5-yl 3-Nitrobenzenesulfonate (4f). To a solution of 5-hydroxyquinoline **10** (20 mg, 0.08 mmol) and Et_3N (50 μL) in CH_2Cl_2 (2 mL) was added 3-nitrobenzene-1-sulfonyl chloride (20 mg, 0.09 mmol). The mixture was stirred overnight, poured into water (5 mL), and extracted with $CHCl_3$ (3 × 5 mL). The organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum, and purified by chromatography ($CHCl_3$/MeOH = 10:1) to give **4f** as a white solid (81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.74 (d, J = 3.3 Hz, 1H), 8.46 (d, J = 9.6 Hz, 1H), 8.11 (d, J = 8.7 Hz, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.68 (t, J = 8.1 Hz, 1H), 7.36 (t, J = 8.7 Hz, 1H), 7.21 (m, 2H), 3.25 (t, J = 4.8 Hz, 4H), 2.58 (t, J = 4.8 Hz, 4H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.2, 144.9, 143.4, 143.0, 137.5, 133.8, 130.6, 128.6, 124.6, 123.5, 123.0, 119.4, 108.8, 54.5, 48.1, 46.0; MS (EI) m/z 428 (M^+); HRMS calcd for $C_{20}H_{20}N_4O_5S$ (M^+) 428.1154, found 428.1157.

General Procedure for Preparation of 3,5-Diaminoquinolines 11a and 11b. 5-Aminoquinoline **9a** or **9b** (0.33 mmol) and 3-nitrobenzaldehyde (0.36 mmol) were mixed in ethanol (3 mL), and the mixture was heated to reflux overnight. The solvent was removed. The crude imino intermediate was dissolved in ethanol (3 mL), and $NaBH_4$ (1.2 mmol) was added. The mixture was stirred at room temperature for 1 h. Acetone (1 mL) was added into the mixture followed by addition of water (5 mL). The reaction mixture was extracted with $CHCl_3$ (3 × 5 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum, and purified by chromatography ($CHCl_3$/MeOH = 10:1) to give the corresponding title compounds **11a** and **11b**.

3-(4-Methylpiperazin-1-yl)-*N*-(3-nitrobenzyl)quinolin-5-amine (11a). Yellow solid (67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (d, J = 2.7 Hz, 1H), 8.24 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 7.44 (m, 3H), 7.25 (m, 1H), 6.46 (d, J = 7.5 Hz, 1H), 4.97 (s, 1H), 4.59 (s, 2H), 3.28 (t, J = 4.8 Hz, 4H), 2.59 (t, J = 4.8 Hz, 4H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 144.1, 143.9, 143.3, 141.6, 141.3, 133.3, 129.5, 126.7, 122.2, 122.0, 119.1, 118.9, 110.2, 106.0, 54.7, 49.0, 47.7, 46.0; MS (EI) m/z 377 (M^+); Anal. ($C_{21}H_{23}N_5O_2 \cdot 1.0HCl \cdot 0.15H_2O$) C, H, N.

3-(4-Acetylpiperazin-1-yl)-*N*-(3-nitrobenzyl)quinolin-5-amine (11b). Yellow solid (59%); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 8.62 (d, J = 2.4 Hz, 1H), 8.20 (s, 1H), 8.04 (d, J = 9.3 Hz, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.56 (s, 1H), 7.44 (t, J = 8.1 Hz, 1H), 7.25 (m, 2H), 6.37 (d, J = 8.4 Hz, 1H), 4.56 (s, 2H), 3.74 (t, J = 5.1 Hz, 2H), 3.64 (t, J = 5.1 Hz, 2H), 3.26 (t, J = 5.1 Hz, 2H), 3.19 (t, J = 5.1 Hz, 2H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 169.7, 148.2, 143.5, 143.2, 143.1, 142.1, 141.5, 133.0, 129.3, 127.5, 121.8, 121.5, 118.7, 116.9, 112.9, 105.4, 49.7, 49.1, 46.9, 45.9, 41.0, 20.6; MS (EI) m/z 405 (M^+). Anal. ($C_{22}H_{23}N_5O_3 \cdot 0.55H_2O$) C, H, N.

General Procedure for Preparation of 3,5-Disubstituted Quinolines 12a−g. To a solution of 5-aminoquinoline **9a** or **9b** (0.1 mmol) in CH_2Cl_2 (2 mL) was added an appropriate sulfonyl chloride, isocyanate, isothiocyanate, or carboxylic acid (0.11 mmol), a base (Et_3N or DIPEA) or a condensation agent TBTU as needed (0.11 mmol). The mixture was stirred at room temperature overnight. The solvent was concentrated in vacuum and the residue was purified by chromatography ($CHCl_3$/MeOH = 10:1) to give corresponding quinolines **12a−g**.

***N*-(3-(4-Methylpiperazin-1-yl)quinolin-5-yl)-3-nitrobenzenesulfonamide (12a).** Yellow powder (52%); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 8.76 (d, J = 2.1 Hz, 1H), 8.42 (s, 1H), 8.38 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.70 (t, J = 8.1 Hz, 2H), 7.36 (m, 3H), 3.46 (s, 1H), 3.14 (t, J = 3.9 Hz, 4H), 2.52 (t, J = 3.9 Hz, 4H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.7, 144.0, 142.3, 142.2, 132.5, 131.7, 131.2, 126.9, 126.6, 125.3, 124.8, 123.7, 121.3, 110.4, 53.9, 47.4, 45.4; MS (EI) m/z 427 (M^+). Anal. ($C_{20}H_{21}N_5O_4S \cdot 0.5H_2O$) C, H, N.

1-(3-(4-Methylpiperazin-1-yl)quinolin-5-yl)-3-(3-nitrophenyl)urea (12b). Yellow powder (82%); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 8.62 (d, J = 2.1 Hz, 1H), 8.24 (t, J = 2.4 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 7.77 (t, J = 7.2 Hz, 2H), 7.66 (d, J = 7.5 Hz, 1H), 7.43 (m, 2H), 7.35 (t, J = 8.4 Hz, 1H), 3.23 (t, J = 4.8 Hz, 4H), 2.60 (t, J = 4.8 Hz, 4H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 153.4, 148.3, 144.4, 144.0, 142.3, 140.2, 131.9, 129.3, 126.3, 124.2, 124.1, 122.7, 120.4, 116.8, 112.9, 111.4, 54.2, 48.1, 45.4; MS (ESI) m/z 407 $(M + H)^+$. Anal. ($C_{21}H_{22}N_6O_3$) C, H, N.

General Procedure for Preparation of 5-Aryl-3-aminoquinolines 14a−f. Iodide **13** (0.1 mmol), an appropriate boronic acid (0.15 mmol), and $Pd_2Cl(dppf)$ (0.02 mmol) were mixed in THF (5 mL). A solution of K_3PO_4 (0.45 mmol) in H_2O (1.5 mL) was added in small portions, and the mixture was stirred for 2 h. The reaction was quenched by pouring into water (10 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuum, and purified by chromatography ($CHCl_3$/MeOH = 30:1), yielding corresponding 5-aryl substituted quinolines **14a−f**.

3-(4-Methylpiperazin-1-yl)-5-(naphthalen-2-yl)quinoline (14a). Yellow solid (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (d, J = 2.1 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.90 (m, 4H), 7.55 (m, 6H), 3.19 (t, J = 5.4 Hz, 4H), 2.59 (t, J = 5.4 Hz, 4H), 2.32 (s, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 144.7, 144.2, 143.0, 138.8, 137.4, 133.4, 132.5, 128.5, 128.4, 128.0, 127.8, 127.6, 127.1, 126.3, 126.1, 125.8, 114.8, 54.6, 48.7, 46.0; MS (EI) *m/z* 353 (M^+). Anal. ($C_{24}H_{23}N_3\cdot0.25H_2O$) C, H, N.

General Procedure for Preparation of 3,5,7-Trisubstituted Quinolines 21a−d,f−l. These compounds were prepared from 5-nitroquinoline **20a−f** through Fe/NH_4Cl reduction followed by reductive amination with an appropriate aldehyde using a procedure similar to the preparation of 3,5-disubstituted quinolines **11a** and **11b**.

3-(4-Methylpiperazin-1-yl)-5-(3-nitrobenzylamino)-7-(trifluoromethyl)quinoline (21a). Yellow powder (70%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.66 (s, 1H), 7.48 (t, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 1.5 Hz, 1H), 6.59 (s, 1H), 5.07 (t, *J* = 5.4 Hz, 1H), 4.59 (d, *J* = 5.4 Hz, 2H), 3.32 (t, *J* = 4.8 Hz, 4H), 2.57 (t, *J* = 4.8 Hz, 4H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 148.3, 144.9, 144.4, 142.8, 141.6, 140.6, 133.3, 129.5, 128.3 (q, *J* = 31.9 Hz), 124.2 (q, *J* = 3270.6 Hz), 122.2, 122.0, 120.6, 115.4, 110.3, 100.3, 54.4, 48.2, 47.1, 45.7; MS (EI) *m/z* 445 (M^+). Anal. ($C_{22}H_{22}F_3N_4O_2\cdot0.40H_2O$) C, H, N.

3-(4-Acetylpiperazin-1-yl)-5-(3-nitrobenzylamino)-7-(trifluoromethyl)quinoline (21b). Yellow powder (64%); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 8.65 (s, 1H), 8.17 (s, 1H), 8.03 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.58 (s, 1H), 7.45 (m, 2H), 6.40 (s, 1H), 4.55 (s, 2H), 3.71 (s, 2H), 3.63 (s, 2H), 3.32 (s, 2H), 3.24 (s, 2H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 147.9, 144.6, 143.8, 142.0, 141.3, 133.9, 130.0, 126.6 (q, *J* = 31.0 Hz), 124.6 (q, *J* = 270.1 Hz), 121.9, 121.7, 120.3, 113.6, 110.4, 98.1, 48.1, 47.6, 45.5, 45.2, 40.4, 21.2; MS (EI) *m/z* 473 (M^+). Anal. ($C_{23}H_{22}F_3N_5O_3\cdot1.0H_2O$) C, H, N.

3-(4-Ethylpiperazin-1-yl)-5-(3-nitrobenzylamino)-7-(trifluoromethyl)quinoline (21c). Yellow powder (65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.27 (s, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.70 (s, 1H), 7.53 (t, *J* = 7.8 Hz, 1H), 7.27 (s, 1H), 6.61 (s, 1H), 4.85 (t, *J* = 5.4 Hz, 1H), 4.63 (d, *J* = 5.4 Hz, 2H), 3.37 (t, *J* = 4.8 Hz, 4H), 2.65 (t, *J* = 4.8 Hz, 4H), 2.49 (q, *J* = 7.2 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 145.1, 144.7, 142.3, 141.8, 140.4, 133.5, 129.7, 128.1 (q, *J* = 32.0 Hz), 124.3 (q, *J* = 270.8 Hz), 122.7, 122.3, 120.6, 117.0, 108.7, 101.0, 52.3, 52.2, 48.5, 47.7, 11.9; MS (EI) *m/z* 459 (M^+). Anal. ($C_{23}H_{24}F_3N_5O_2\cdot0.10H_2O$) C, H, N.

***tert*-Butyl 4-(5-(3-Nitrobenzylamino)-7-(trifluoromethyl)-quinolin-3-yl)piperazine-1-carboxylate (21d).** Yellow powder (68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (d, *J* = 2.7 Hz, 1H), 8.28 (s, 1H), 8.15 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.71 (s, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 2.1 Hz, 1H), 6.63 (s, 1H), 4.94 (t, *J* = 5.1 Hz, 1H), 4.64 (d, *J* = 5.1 Hz, 2H), 3.64 (t, *J* = 5.1 Hz, 4H), 3.28 (t, *J* = 5.1 Hz, 4H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 148.3, 145.1, 145.0, 142.7, 142.2, 140.3, 133.7, 129.7, 128.5 (q, *J* = 31.9 Hz), 124.2 (q, *J* = 270.5 Hz), 122.5, 122.2, 120.4, 116.4, 110.1, 100.8, 80.2, 48.7, 47.6, 43.5, 28.3; MS (EI) *m/z* 531 (M^+). Anal. ($C_{26}H_{28}F_3N_5O_4$) C, H, N.

***N*-(3-(4-Methylpiperazin-1-yl)-7-(trifluoromethyl)quinolin-5-yl)-3-nitrobenzenesulfonamide (22).** This compound was prepared as a yellow solid from **20a** in 85% yield by following a similar procedure as that for preparation of disubstituted quinoline **4f**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (d, *J* = 2.7 Hz, 1H), 8.46 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.92 (s, 1H), 7.82 (d, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.14 (s, 1H), 3.19 (d, *J* = 5.4 Hz, 4H), 2.51 (d, *J* = 5.4 Hz, 4H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 147.8, 145.4, 144.6, 141.1, 140.7, 132.4, 131.8, 130.1, 127.7, 126.8, 124.3, 121.8, 120.0, 111.0, 53.8, 47.0, 45.1; MS (EI) *m/z* 495 (M^+); HRMS calcd for $C_{21}H_{20}F_3N_5O_4S$ (M^+) 495.1188, found 495.1193.

3-(4-Methylpiperazin-1-yl)-5-(3-nitrobenzyloxy)-7-(trifluoromethyl)quinoline (24). This compound was prepared as a yellow solid (90%) from phenol **23** following a similar procedure as that for preparation of compounds **4a−c**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (d, *J* = 2.7 Hz, 1H), 8.46 (s, 1H), 8.24 (d, *J* = 9.3 Hz, 1H), 7.93 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.73 (d, *J* = 2.4 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 6.97 (s, 1H), 5.36 (s, 2H), 3.41 (t, *J* = 4.8 Hz, 4H), 2.64 (t, *J* = 4.8 Hz, 4H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 148.5, 145.5, 145.2, 141.8, 138.3, 133.0, 129.7, 127.2, 123.2, 123.0, 122.1, 120.0, 110.0, 101.7, 69.2, 54.6, 48.2, 46.1; MS (EI) *m/z* 446 (M^+); HRMS calcd for $C_{22}H_{21}F_3N_4O_3$ (M^+) 446.1566, found 446.1565.

General Procedure for Preparation of 3,5,7-Trisubstituted Quinolines 27a−d. These compounds were prepared by reduction of 5-nitroquinolines **26a−d** with Fe/NH_4Cl followed by reductive amination via a procedure similar to that of preparation of quinolines **11a** and **11b**.

3-(4-(Cyclopropylsulfonyl)piperazin-1-yl)-*N*-(3-nitrobenzyl)-7-(trifluoromethyl)quinolin-5-amine (27a). Yellow solid (60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (d, *J* = 1.8 Hz, 1H), 8.30 (s, 1H), 8.15 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.70 (s, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 1.5 Hz, 1H), 6.62 (s, 1H), 5.19 (t, *J* = 5.1 Hz, 1H), 4.67 (d, *J* = 5.1 Hz, 2H), 3.53 (t, *J* = 4.8 Hz, 4H), 3.39 (t, *J* = 4.8 Hz, 4H), 2.32 (s, 1H), 1.20 (m, 2H), 1.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 148.4, 145.0, 144.5, 143.0, 142.1, 140.6, 133.3, 129.6, 129.1 (q, *J* = 31.9 Hz), 124.1 (q, *J* = 270.6 Hz), 122.4, 121.9, 120.3, 115.2, 111.7, 100.5, 49.5, 47.0, 45.7, 25.3, 4.2; MS (EI) *m/z* 535 (M^+). Anal. ($C_{24}H_{24}F_3N_5O_4S$) C, H, N.

3-(4-(Methylsulfonyl)piperazin-1-yl)-*N*-(3-nitrobenzyl)-7-(trifluoromethyl)quinolin-5-amine (27b). Yellow solid (62%); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 8.70 (d, *J* = 2.4 Hz, 1H), 8.22 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.70 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 2.7 Hz, 1H), 7.55 (s, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 6.48 (s, 1H), 4.59 (s, 2H), 3.40 (s, 8H), 2.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.9, 144.5, 144.2, 143.9, 142.0, 141.4, 133.8, 130.0, 126.8 (q, *J* = 30.9 Hz), 124.6 (q, *J* = 270.5 Hz), 122.0, 121.7, 120.2, 113.5, 110.9, 98.1, 47.5, 45.4, 45.0, 33.9; MS (EI) *m/z* 509 (M^+). Anal. ($C_{22}H_{22}F_3N_5O_4S$) C, H, N.

***N*-(3-Nitrobenzyl)-5-(trifluoromethyl)quinolin-7-amines 31a and 31b.** These two compounds were prepared from anilines **30a** and **30b** following a procedure similar to that of preparation of quinolines **11a** and **11b**. For quinoline **31a**: yellow solid (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.17 (s, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.41 (m, 2H), 7.32 (s, 1H), 6.99 (s, 1H), 4.94 (t, *J* = 5.1 Hz, 1H), 4.48 (d, *J* = 5.1 Hz, 2H), 3.23 (s, 4H), 2.58 (s, 4H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 145.3, 144.7, 144.1, 143.0, 140.6, 133.0, 129.5, 125.6 (q, *J* = 30.0 Hz), 124.0 (q, *J* = 272.0 Hz), 122.3, 121.7, 118.2, 117.5, 114.0, 109.6, 54.6, 49.0, 47.1, 45.9; MS (EI) *m/z* 445 (M^+). Anal. ($C_{22}H_{22}F_3N_5O_2\cdot0.2H_2O$) C, H, N. For quinoline **31b**: yellow solid (69%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, *J* = 2.4 Hz, 1H), 8.20 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.45 (m, 2H), 7.33 (d, *J* = 2.1 Hz, 1H), 7.01 (s, 1H), 4.78 (t, *J* = 4.8 Hz, 1H), 4.52 (d, *J* = 5.7 Hz, 2H), 3.26 (t, *J* = 4.8 Hz, 4H), 2.64 (t, *J* = 4.8 Hz, 4H), 2.48 (q, *J* = 6.9 Hz, 2H), 1.12 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 145.4, 144.7, 144.0, 143.2, 140.6, 133.1, 129.6, 125.6 (q, *J* = 30.1 Hz), 124.0 (q, *J* = 272.4 Hz), 122.4, 121.8, 118.2, 117.5, 114.1, 109.7, 52.4, 52.2, 49.1, 47.2, 11.8; MS (EI) *m/z* 459 (M^+). Anal. ($C_{23}H_{24}F_3N_5O_2$) C, H, N.

ELISA Kinase Assay. The tyrosine kinase activities were evaluated according to the reported protocol.[44−48] Briefly, in an enzyme-linked-immunosorbent assay (ELISA), 20 μg/mL poly(Glu,Tyr) 4:1 (Sigma) was precoated as a substrate in 96-well plates. Then 50 μL of 10 μM ATP solution diluted in kinase reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.2 mM Na_3VO_4, 1 mM DTT) was added to each well. Various concentrations of compounds diluted in 10 μL of 1% DMSO (v/v) were added to each reaction well, with 1% DMSO (v/v) used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 40 μL of kinase reaction buffer solution. After incubation for 60 min at 37 °C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Next, 100 μL of anti-phosphotyrosine (PY99) antibody (1:500 diluted in 5 mg/mL BSA T-PBS) was added. After 30 min of incubation at 37 °C, the plate was washed three times. A solution of 100 μL of horseradish peroxidase-conjugated goat anti-mouse IgG (1:2000 diluted in 5 mg/mL BSA T-PBS) was added. The plate was reincubated at 37 °C for 30 min and washed as before. Finally, 100 μL of a solution containing 0.03% H_2O_2 and 2 mg/mL *o*-phenylenediamine in 0.1 mM citrate buffer, pH 5.5, was added and samples were incubated at room temperature until color emerged. The reaction was terminated by the addition of 50 μL of 2 M H_2SO_4, and the plate was read using a multiwell spectrophotometer (VERSAmax, Molecular Devices, Sunnyvale, CA, U.S.) at 490 nm. The inhibition rate (%) was calculated using the following equation: % inhibition = $[1 - (A_{490}/A_{490\,\mathrm{control}})] \times 100$. IC_{50} values were calculated from the inhibition curves.

Western Blot Analysis. Cells were cultured under regular growth conditions to exponential growth phase. Then the cells were treated with indicated concentration of corresponding compounds for 4 h at 37 °C and lysed in 1× SDS sample buffer. Those cell lysates were subsequently resolved on 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed with, phospho-c-Met and c-Met, phospho-ERK1/2 and ERK1/2, phospho-AKT and AKT (all from Cell Signaling Technology, Beverly, MA) and GAPDH (KangChen Biotech) antibody and then subsequently with anti-rabbit or anti-mouse IgG horseradish peroxidase. Immunoreactive proteins were detected using an enhanced chemiluminescence detection reagent.

Cell Proliferation Assay. Cells were seeded in 96-well tissue culture plates. On the next day, cells were exposed to various concentrations of compounds and further cultured for 72 h. Finally, cell proliferation was determined using sulforhodamine B (SRB, Sigma) or thiazolyl blue tetrazolium bromide (MTT, Sigma) assay.

In Vivo Antitumor Activity Assay. Animal experiments were performed according to institutional ethical guidelines of animal care. The cells at a density of $(5-10) \times 10^6$ in 200 μL were first implanted sc into the right flank of each nude mice and then allowed to grow to 700−800 mm^3, defined as a well-developed tumor. After that, the well-developed tumors were cut into 1 mm^3 fragments and transplanted sc into the right flank of nude mice using a trocar. When the tumor volume reached 100−150 mm^3, the mice were randomly assigned into control and treatment groups. Control groups were given vehicle alone, and treatment groups received quinoline **21b** as indicated doses via ip administration 7 days per week for 2−3 weeks. The sizes of the tumors were measured twice per week using microcaliper. The tumor volume (TV) was calculated as: TV = (length × width^2)/2. Tumor volume was shown on indicated days as the median tumor volume ± SE indicated for groups of mice. Percent (%) inhibition values were measured on the final day of study for drug-treated compared with vehicle-treated mice and are calculated as 100 × {1 − [(treated final day − treated day 1)/(control final day − control day 1)]}. Significant differences between the treated versus the control groups ($P \leq 0.001$) were determined using Student's *t* test.

Reversibility Assessment for 21b. Rapid dilution experiments were used to demonstrate reversible binding of **21b** to c-Met. Then 9 μL of 100-fold normal amount of enzyme (450 nM) was mixed with 1 μL of **21b** at a final concentration of 100-fold IC_{50} for each enzyme or with vehicle control. After incubation at room temperature for 30 min, 1 μL of the mix solution was diluted into 99 μL of a solution containing fluorescent substrate peptide (sequence 5-FAM-EAIYAAPFAKKK-CONH2, 1.5 μM) and ATP (75 μM). The microplate was placed in the EZ reader II, and wells were repeatedly sampled for 180 min. The product (i.e., the phosphorylated substrate) and substrate migrate at different rates through the electrophoretic separation channel. And then, both the substrate and product were detected directly via LED induced fluorescence (LIF). In this case, the fraction of peptide converted to the phosphorylated form here reflects the enzyme activity.

■ ASSOCIATED CONTENT

S Supporting Information. Experimental details for the intermediates and final compounds and their ^{1}H and ^{13}C spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

RTK, receptor tyrosine kinase; HGF, hepatocyte growth factor; ATP, adenosine 5′-triphosphate; EGFR, epidermal growth factor receptor; PDGFRβ, platelet-derived growth factor receptor β; FGFR1, fibroblast growth factor receptor 1; ABL1, Abelson murine leukemia viral oncogene homologue 1; IGF1R, insulin-like growth factor receptor 1; VEGFR2, vascular endothelial cell growth factor receptor 2; IGFR, insulin-like growth factor receptor; ip, intraperitoneal injection; iv, intravenous; PK, pharmacokinetic; RON, recepteur d'origine nantais; SAR, structure−activity relationship; HTS, high throughput screening

■ REFERENCES

(1) Trusolino, L.; Comoglio, P. M. Scatter-factor and semaphoring receptor: cell signaling for invasive growth. *Nat. Rev. Cancer* **2002**, *2*, 289–300.

(2) Birchmeier, C.; Birchmeier, W.; Gherardi, E.; Vande Woude, G. F. Met, metastasis, motility and more. *Nature Rev. Mol. Cell Biol.* **2003**, *4*, 915–925.

(3) Lokker, N. A.; Mark, M. R.; Luis, E.; Bennett, G. L.; Robbins, K. A.; Baker, J. B.; Godowski, P. J. Structure−function analysis of hepatocyte growth factor: identification of variants that lack mitogenic activity yet retain high affinity receptor binding. *EMBO J.* **1992**, *11*, 2503–2510.

(4) Okigaki, M.; Komada, M.; Uehara, Y.; Miyazawa, K.; Kitamura, N. Functional characterization of human hepatocyte growth factor mutants obtained by deletion of structural domains. *Biochemistry* **1992**, *31*, 9555–9561.

(5) Aguirre Ghiso, J. A.; Alonso, D. F.; Farías, E. F.; Gomez, D. E.; de Kier Joffé, E. B. Deregulation of the signaling pathways controlling

urokinase production. Its relationship with the invasive phenotype. *Eur. J. Biochem.* **1999**, *263*, 295–304.
(6) Parr, C.; Watkins, G.; Mansel, R. E.; Jiang, W. G. The hepatocyte growth factor regulatory factors in human breast cancer. *Clin. Cancer Res.* **2004**, *10*, 202–211.
(7) Rong, S.; Segal, S.; Anver, M.; Resau, J. H.; Vande Woude, G. F. Invasiveness and metastasis of NIH 3T3 cells induced by Met-hepatocyte growth factor/scatter factor autocrine stimulation. *Proc. Natl Acad. Sci. U.S.A.* **1994**, *91*, 4731–4735.
(8) Bellusci, S.; Moens, G.; Gaudino, G.; Comoglio, P.; Nakamura, T.; Thiery, J. P.; Jouanneau, J. Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity. *Oncogene* **1994**, *9*, 1091–1099.
(9) Jeffers, M.; Rong, S.; Anver, M.; Vande Woude, G. F. Autocrine hepatocyte growth factor/scatter factor signalling induces transformation and the invasive/metastatic phenotype in C127 cells. *Oncogene* **1996**, *13*, 853–861.
(10) Takayama, H.; LaRochelle, W. J.; Sharp, R.; Otsuka, T.; Kriebel, P.; Anver, M.; Aaronson, S. A.; Merlino, G. Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor. *Proc. Natl Acad. Sci. U.S.A.* **1997**, *94*, 701–706.
(11) Otsuka, T.; Takayama, H.; Sharp, R.; Celli, G.; LaRochelle, W. J.; Bottaro, D. P.; Ellmore, N.; Vieira, W.; Owens, J. W.; Anver, M.; Merlino, G. c-Met autocrine activation induces development of malignant melanoma and acquisition of the metastatic phenotype. *Cancer Res.* **1998**, *58*, 5157–5167.
(12) Cui, J. Inhibitors targeting hepatocyte growth factor receptor and vascular endothelial growth factor receptor tyrosine kinases. *Expert Opin. Ther. Pat.* **2006**, *16*, 713–718.
(13) Cui, J. J. Inhibitors targeting hepatocyte growth factor receptor and their potential therapeutic applications. *Expert Opin. Ther. Pat.* **2007**, *17*, 1035–1045.
(14) Abidoye, O.; Murukurthy, N.; Salgia, R. Review of clinic trials: agents targeting c-Met. *Rev. Recent Clin. Trials.* **2007**, *2*, 143–147.
(15) Comoglio, P. M.; Giordano, S.; Trusolino, L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat. Rev. Drug Discovery* **2008**, *7*, 504–516.
(16) Liu, X.; Yao, W.; Newton, R. C.; Scherle, P. A. Targeting the c-Met signaling pathway for cancer therapy. *Expert Opin. Invest. Drugs* **2008**, *17*, 997–1011.
(17) Dussault, I.; Bellon, S. F. From concept to reality: the long road to c-Met and RON receptor tyrosine kinase inhibitors for the treatment of cancer. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 221–229.
(18) Eder, J. P.; Vande Woude, G. F.; Boerner, S. A.; LoRusso, P. M. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. *Clin. Cancer Res.* **2009**, *15*, 2207–2214.
(19) Giubellino, A.; Linehan, W. M.; Bottaro, D. P. Targeting the Met signaling pathway in renal cancer. *Expert Rev. Anticancer Ther.* **2009**, *9*, 785–793.
(20) Underiner, T. L.; Herbertz, T.; Miknyoczki, S. J. Discovery of small molecule c-Met inhibitors: evolution and profiles of clinical candidates. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 7–27.
(21) Porter, J. Small molecular c-Met kinase inhibitors: a review of recent patent. *Expert Opin. Ther. Pat.* **2010**, *20*, 159–177.
(22) Zou, H. Y.; Li, Q.; Lee, J. H.; Arango, M. E.; McDonnell, S. R.; Yamazaki, S.; Koudriakova, T. B.; Alton, G.; Cui, J. J.; Kung, P. P.; Nambu, M. D.; Los, G.; Bender, S. L.; Mroczkowski, B.; Christensen, J. G. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res.* **2007**, *67*, 4408–4417.
(23) Wang, X.; Le, P.; Liang, C.; Chan, J.; Kiewlich, D.; Miller, T.; Harris, D.; Sun, L.; Rice, A.; Vasile, S.; Blake, R. A.; Howlett, A. R.; Patel, N.; McMahon, G.; Lipson, K. E. Potent and selective inhibitors of the Met [hepatocyte growth factor/scatter factor (HGF/SF) receptor] tyrosine kinase block HGF/SF-induced tumor cell growth and invasion. *Mol. Cancer Ther.* **2003**, *2*, 1085–1092.
(24) Fujiwara, Y.; Senga, T.; Nishitoba, T.; Osawa, T.; Miwa, A.; Nakamura, K. Quinoline Derivative and Quinazoline Derivative Inhibiting Self-Phosphorylation of Hepatocytus Proliferator Receptor, and Medicinal Composition Containing the Same. PCT Int. Appl. WO2003000660A1, 2003.
(25) Porter, J.; Lumb, S.; Lecomte, F.; Reuberson, J.; Foley, A.; Calmiano, M.; le Riche, K.; Edwards, H.; Delgado, J.; Franklin, R. J.; Gascon-Simorte, J. M.; Maloney, A.; Meier, C.; Batchelor, M. Discovery of a novel series of quinoxalines as inhibitors of c-Met kinase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 397–400.
(26) Ding, C. Y.; Tu, S. H.; Yao, Q. Z.; Li, F. L.; Wang, Y. X.; Hu, W. X.; Zhang, A. One-pot three-step synthesis of naphtho[2,3-*a*]carbazole-5,13-diones using tandem radical alkylation−cyclization−aromatization reaction sequence. *Adv. Synth. Catal.* **2010**, *352*, 847–853.
(27) Ding, C. Y.; Tu, S. H.; Li, F. Y.; Wang, Y. X.; Yao, Q. Z.; Hu, W. X.; Xie, H.; Meng, L. H.; Zhang, A. Synthesis study on marmycin A: preparation of the C3′-desmethyl analogues. *J. Org. Chem.* **2009**, *74*, 6111–6119.
(28) Hartley, C. S.; Elliott, E. L.; Moore, J. S. Covalent assembly of molecular ladders. *J. Am. Chem. Soc.* **2007**, *129*, 4512–4513.
(29) Wolfe, J. P.; Buchwald, S. L. Scope and limitations of the Pd/BINAP-catalyzed amination of aryl bromid. *J. Org. Chem.* **2000**, *65*, 1144–1157.
(30) Bromidge, S. M.; Griffith, K.; Heightman, T. D.; Jennings, A.; King, F. D.; Moss, S. F.; Newman, H.; Riley, G.; Routledge, C.; Serafinowska, H. T.; Thomas, D. R. Novel (4-piperazin-1-ylquinolin-6-yl)arylsulfonamides with high affinity and selectivity for the 5-HT(6) receptor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2843–2846.
(31) Kim, K. S.; Zhang, L.; Schmidt, R.; Cai, Z. W.; Wei, D.; Williams, D. K.; Lombardo, L. J.; Trainor, G. L.; Xie, D.; Zhang, Y.; An, Y.; Sack, J. S.; Tokarski, J. S.; Darienzo, C.; Kamath, A.; Marathe, P.; Zhang, Y.; Lippy, J.; Jeyaseelan, R., Sr.; Wautlet, B.; Henley, B.; Gullo-Brown, J.; Manne, V.; Hunt, J. T.; Fargnoli, J.; Borzilleri, R. M. Discovery of pyrrolopyridine−pyridone based inhibitors of Met kinase: synthesis, X-ray crystallographic analysis, and biological activities. *J. Med. Chem.* **2008**, *51*, 5330–5341.
(32) Kato, T.; Okamoto, I.; Tanatani, A.; Hatano, T.; Uchiyama, M.; Kagechika, H.; Masu, H.; Katagiri, K.; Tominaga, M.; Yamaguchi, K.; Azumaya, I. Spontaneous resolution of aromatic sulfonamides: effective screening method and discrimination of absolute structure. *Org. Lett.* **2006**, *8*, 5017–5020.
(33) Casey, C. P.; Johnson, J. B. Isomerization and deuterium scrambling evidence for a change in the rate-limiting step during imine hydrogenation by Shvo's hydroxycyclopentadienyl ruthenium hydride. *J. Am. Chem. Soc.* **2005**, *127*, 1883–1894.
(34) Ramamurthy, B.; Bhatt, M. V. Synthesis and antitubercular activity of *N*-(2-naphthyl)glycine hydrazide analogues. *J. Med. Chem.* **1989**, *32*, 2421–2426.
(35) Kung, P. P.; Funk, L.; Meng, J.; Alton, G.; Padrique, E.; Mroczkowski, B. Structure activity relationships of quinoline-containing c-Met inhibitors. *Eur. J. Med. Chem.* **2008**, *43*, 1321–1329.
(36) Loones, K. T.; Maes, B. U.; Dommisse, R. A. Synthesis of pyrido[2′,1′:2,3] imidazo[4,5-*b*]quinoline and pyrido[1′,2′:1,2]imidazo[4,5-*b*]quinoline and their benzo and aza analogs via tandem catalysis. *Tetrahedron* **2007**, *63*, 8954–8961.
(37) Coudret, C.; Mazenc, V. Heteroarylation of anthraquinone-triflate by Suzuki cross-coupling. *Tetrahedron Lett.* **1997**, *38*, 5293–5296.
(38) Belcher, B. R.; Stacey, M.; Skyes, A.; Tatlow, J. C. The synthesis of certain trifluoromethylquinoline derivatives. *J. Chem. Soc.* **1954**, 3846–3851.
(39) Jones, G. Synthesis of the Quinoline Ring System. In *Quinolines*; Jones, G., Ed.; Wiley: New York, 1977; Chapter 2.
(40) Reitsema, R. H. The chemistry of 4-hydroxyquinolines. *Chem. Rev.* **1948**, *43*, 43–68.
(41) Manske, R. H. The chemistry of isoquinolines. *Chem. Rev.* **1942**, *30*, 145–158.
(42) Denmark, S. E.; Venkatraman, S. On the mechanism of the Skraup−Doebner−Von Miller quinoline synthesis. *J. Org. Chem.* **2006**, *71*, 1668–1676.

(43) Henry, R. A.; Moore, D. W.; Atkins, R. L. 5-Trifluoromethyl-1,7-phenanthroline. *J. Heterocycl. Chem.* **1976**, *13*, 375–376.

(44) D'Angelo, N. D.; Bellon, S. F.; Booker, S. K.; Cheng, Y.; Coxon, A.; Dominguez, C.; Fellows, I.; Hoffman, D.; Hungate, R.; Kaplan-Lefko, P.; Lee, M. R.; Li, C.; Liu, L.; Rainbeau, E.; Reider, P. J.; Rex, K.; Siegmund, A.; Sun, Y.; Tasker, A. S.; Xi, N.; Xu, S.; Yang, Y.; Zhang, Y.; Burgess, T. L.; Dussault, I.; Kim, T. S. Design, synthesis, and biological evaluation of potent c-Met inhibitors. *J. Med. Chem.* **2008**, *51*, 5766–5779.

(45) Albrecht, B. K.; Harmange, J. C.; Bauer, D.; Berry, L.; Bode, C.; Boezio, A. A.; Chen, A.; Choquette, D.; Dussault, I.; Fridrich, C.; Shimanovich, R.; Springer, S. K.; Teffera, Y.; Yang, Y.; Zhang, Y.; Bellon, S. F. Discovery and optimization of triazolopyridazines as potent and selective inhibitors of the c-Met kinase. *J. Med. Chem.* **2008**, *51*, 2879–2882.

(46) Liu, L.; Siegmund, A.; Xi, N.; Kaplan-Lefko, P.; Rex, K.; Bak, A.; Dominguez, C.; Norman, M. H.; Harmange, J. C.; Dussault, I.; Kim, T. S. Discovery of a potent, selective, and orally bioavailable c-Met inhibitor: 1-(2-hydroxy-2-methylpropyl)- *N*-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamide (AMG 458). *J. Med. Chem.* **2008**, *51*, 3688–3691.

(47) Schroeder, G. M.; An, Y.; Cai, Z. W.; Wautlet, B. S.; Wei, D.; Williams, D. K.; Zhang, Y.; Zhang, Y. P.; Fargnoli, J.; Borzilleri, R. M. Discovery of *N*-(4-(2-Amino-3-chloropyridin-4-yloxy)-3-fluorophenyl)-4-ethoxy-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (BMS-777607), a selective and orally efficacious inhibitor of the Met kinase superfamily. *J. Med. Chem.* **2009**, *52*, 1251–1254.

(48) Peach, M. L.; Tan, N.; Choyke, S. J.; Giubellino, A.; Athauda, G.; Burke, T. R., Jr.; Nicklaus, M. C.; Bottaro, D. P. Directed discovery of agents targeting the Met tyrosine kinase domain by virtual screening. *J. Med. Chem.* **2009**, *52*, 943–951.

(49) Park, M.; Dean, M.; Cooper, C. S.; Schmidt, M.; O'Brien, S. J.; Blair, D. G.; Vande Woude, G. F. Mechanism of met oncogene activation. *Cell* **1986**, *45*, 895–904.

(50) Sattler, M.; Pride, Y. B.; Ma, P.; Gramlich, J. L.; Chu, S. C.; Quinnan, L. A.; Shirazian, S.; Liang, C.; Podar, K.; Christensen, J. G.; Salgia, R. A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase. *Cancer Res.* **2003**, *63*, 5462–5469.

(51) Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. A unique structure for epidermal growth factor receptor bound to GW572016 (lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.* **2004**, *64*, 6652–6659.

(52) Bertotti, A.; Burbridge, M. F.; Gastaldi, S.; Galimi, F.; Torti, D.; Medico, E.; Giordano, S.; Corso, S.; Rolland-Valognes, G.; Lockhart, B. P.; Hickman, J. A.; Comoglio, P. M.; Trusolino, L. Only a subset of Met-activated pathways are required to sustain oncogene addiction. *Sci. Signaling* **2009**, *2*, ra80.