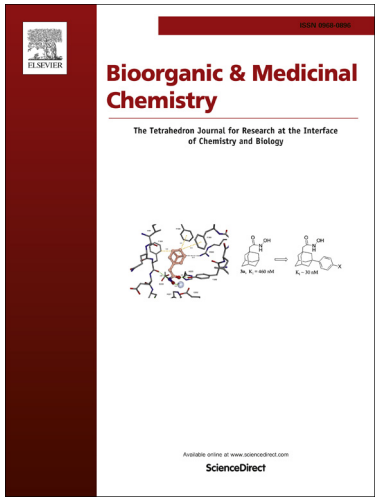
Accepted Manuscript



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4-Quinolone-3-carboxylic Acids as Cell-permeable Inhibitors of Protein Tyrosine Phosphatase 1B

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Abstract: Protein tyrosine phosphatase 1B is a negative regulator in the insulin and leptin signaling pathways, and has emerged as an attractive target for the treatment of type 2 diabetes and obesity. However, the essential pharmacophore of charged phosphotyrosine or its mimetic confer low selectivity and poor cell permeability. Starting from our previously reported aryl diketoacid-based PTP1B inhibitors, a drug-like scaffold of 4-quinolone-3-carboxylic acid was introduced for the first time as a novel surrogate of phosphotyrosine. An optimal combination of hydrophobic groups installed at C-6, N-1 and C-3 positions of the quinolone motif afforded potent PTP1B inhibitors with low micromolar IC_{50} values. These quinolone-3-carboxylate based PTP1B inhibitors displayed a 2-10 fold selectivity over a panel of PTP's. Furthermore, the bidentate inhibitors of 4-quinolone-3-carboxylic acids conjugated with aryl diketoacid or salicylic acid were cell permeable and enhanced insulin signaling in CHO/*h*IR cells. The kinetic studies and molecular modeling suggest that the quinolone-3-carboxylates act as competitive inhibitors by binding to the PTP1B active site in the WPD loop closed conformation. Taken together, our study shows that the 4-quinolone-3-carboxylic acid derivatives exhibit improved pharmacological properties over previously described PTB1B inhibitors and warrant further preclinical studies.

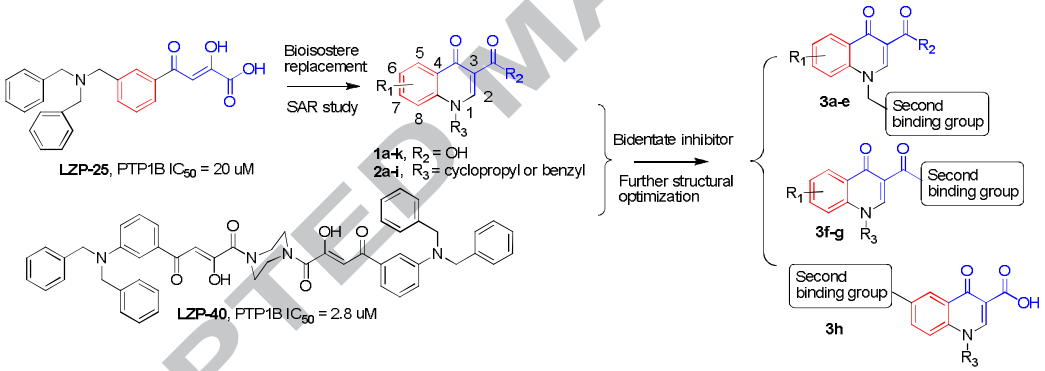
Key words: protein tyrosine phosphatase 1B inhibitor; quinolone-3-carboxylic acid; bidentate ligand; bioisostere; insulin receptor signaling

Introduction

The rapidly increased incidence of type 2 diabetes mellitus (T2DM) and obesity in the population worldwide has fueled an intensified search for new therapeutic treatment options.[1, 2] Insulin resistance is a major pathophysiological factor in the development of T2DM, occurring mainly in muscles, adipose tissues, and liver, leading to reduced glucose uptake and utilization and increased glucose production, respectively.[3, 4] The protein tyrosine phosphatase 1B (PTP1B) is a crucial negative regulator in signal transduction for both insulin and leptin pathways,[5, 6] by dephosphorylating and inactivating the insulin receptor (IR),[7] insulin receptor substrates (IRS)[8] as well as Janus kinase 2 (JAK2).[9, 10] Two independent studies of PTP1B knockout mice have revealed phenotypes of enhanced insulin sensitivity, improved glycemic control, and resistance to high fat diet induced obesity. Interestingly, the PTP1B deficient mice still have normal development and longevity.[11, 12] Furthermore, treatment of diabetic mice with PTP1B antisense oligonucleotides reduced the expression level of the enzyme and subsequently normalized blood glucose and improved insulin sensitivity.[13] Therefore, PTP1B has emerged as a promising and a validated therapeutic target to effectively treat T2DM and obesity.[14, 15] Accordingly, small molecule inhibitors of this enzyme have been pursued extensively by both industry and academia.[16-18] However, only two small molecule PTP1B inhibitors entered clinical trials.[18, 19] Poor bioavailability and low selectivity of negatively charged phosphotyrosine mimetics are the major concerns of the many previously disclosed PTP1B inhibitors.[20] More significantly, because of the structural homologies in PTPs, designing selective PTP1B inhibitors has remained elusive.

Previously, we identified aryl diketoacid chemotype as a new class of PTP1B noncompetitive inhibitors that target the enzyme inactive conformation, conferring excellent selectivity and cellular efficacy.[21] Our compounds are distinctly different from most reported PTP1B inhibitors that are active site-directed competitive inhibitors.[16, 18] Crystal structures of ligand-bound PTP1B reveal that the aryl diketoacid derivatives bind to the PTP1B active site. Remarkably, they bind to the

catalytically inactive, WPD loop open conformation, thus providing a unique opportunity for creating active site-directed PTP1B inhibitors with improved pharmacological properties. Because some diketoacids are biologically labile, we decided to develop novel bioavailable PTP1B inhibitors using bioisostere replacement strategy. 4-Quinolone-3-carboxylic acid has been reported as an effective bioisostere of the diketoacid pharmacophore in the HIV-1 integrase inhibitor design.[22, 23] Also, this scaffold is present in various bioactive compounds and several approved drugs.[24] Therefore, starting from the aryl diketoacid PTP1B inhibitor **LZP-25**,[21] we introduced for the first time the 4-quinolone-3-carboxylic acid scaffold into the design of novel PTP1B inhibitors for enhanced potency and properties. As depicted in **Scheme 1**, the substitution on the quinolone motif was optimized to generate bidentate inhibitors, affording a new class of selective and cell-permeable PTP1B inhibitors bearing the 4-quinolone-3-carboxyl motif.



Scheme 1. The design of 4-quinolone-3-carboxylic acid-based PTP1B inhibitors with investigations on the substitution effect and the conjugation pattern of the bidentate inhibitor

Inhibitor Design

Previously, we showed that the hydrophobic group was essential for the PTP1B inhibition by the aryl diketoacid lead compound **LZP-25**.[21] Therefore, the new series of quinolones were designed bearing the same hydrophobic moiety. Our initial SAR study on the 4-quinolone-3-carboxylate-based PTP1B inhibitors was focused on the appropriate installation of a hydrophobic moiety and the neutralization of the

carboxylic acid for a better bioavailability. Thus, the substituent effect of the quinolone motif on the PTP1B inhibitory activity was investigated, with respect to the substituents on the phenyl ring, the 3-carboxyl derivatization and 1-N substituent (Scheme 1, compounds **1a-k** and **2a-i**).

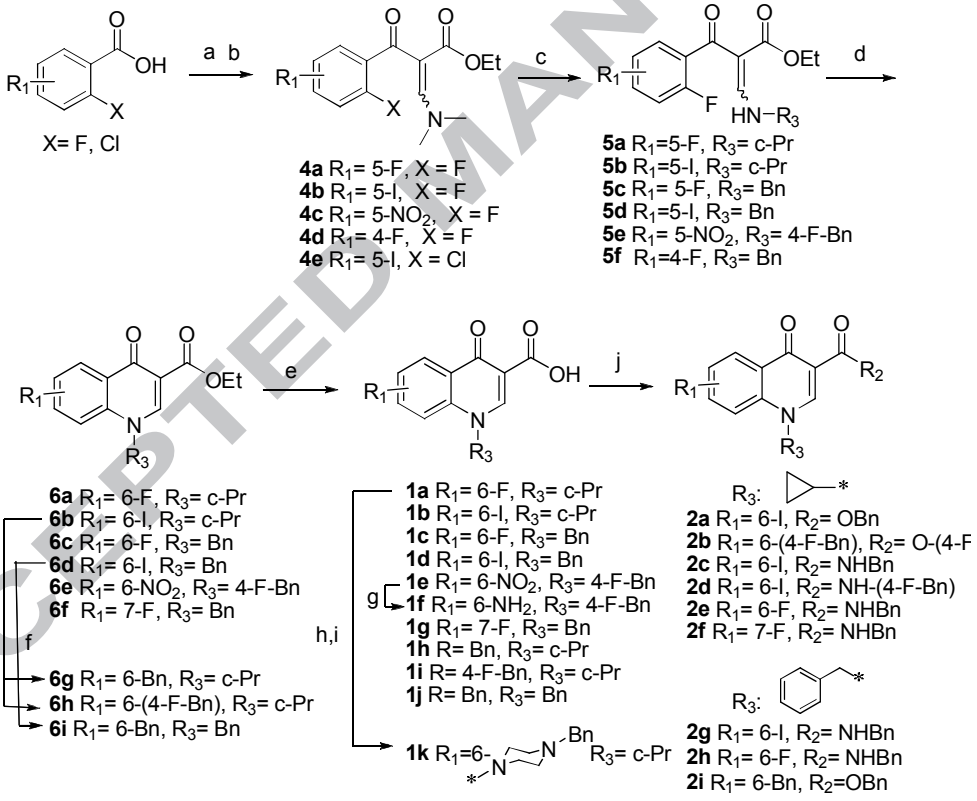
Inspired by the previous finding that the dimerization of the aryl diketoacid through an amide linkage remarkably improved the PTP1B inhibitory activity (**LZP-40,** IC_{50} = 2.8 μM *vs.* **LZP-25**, IC_{50} = 20 μM),[21] we further explored 3-carboxylquinolone-containing bidentate PTP1B inhibitors. To achieve an optimal conjugation and thus gain higher potency and selectivity, three types of bifunctional molecules were designed by conjugating a salicylic acid or an aryl diketoacid functionality with the 4-quinolone-3-carboxylic acid at N-1, C-3 or C-6 position (**Scheme 1**, compounds **3a-h**). For the PTP1B enzyme, a secondary non-catalytic arylphosphate-binding site adjacent to the active site was also identified representing an important structural feature of this phosphatase.[25] The salicylic acid moiety was reported to facilitate the interaction with the second binding site of PTP1B pocket.[26] Taken together, two classes of novel quinolone-3-carboxy-based PTP1B inhibitors, *i.e.* substituted monomers and bidentate ligands, were designed and synthesized as shown in Scheme 1.

Synthesis

The variously substituted quinolone-3-carboxylic acid derivatives (**1a-k, 2a-i**) were synthesized according to a known procedure[22] based on Grohe-Heitzer reaction,[27] as depicted in **Scheme 2**. Starting from the substituted benzoic acid, the acid chloride was condensed with ethyl 3-(dimethylamino)acrylate to produce the enaminoester **4**. After the substituent exchange at N-1 position from dimethyl to benzyl or cyclopropyl group, the intramolecular S_NAr reaction of the halo substituted phenylenamine **5** with potassium carbonate gave the key intermediate quinolone (**6a-f**). Further substituents on the phenyl ring was introduced by Negishi coupling of 6-iodide quinolone with aryl zinc bromides (**6g-i**). Hydrolysis of these quinolone-3-carboxylates (**6a-i**) with HCl (6N) in MeOH at reflux produced the target

compounds (**1a-e**, **1g-j**). Hydrogenolysis catalyzed by Pd-C converted the 6-nitro substituted quinolone-3-carboxylic acid (**1e**) into the 6-amino analog (**1f**). Nucleophilic substitution of the 6-fluoro substituted quinolone-3-carboxylic acid **1a** by piperazine in NMP under microwave irradiation followed by reductive amination with benzaldehyde delivered compound **1k**. Further coupling of these substituted quinolone-3-caroxylic acids with various amines by BOP and Et_3N readily generated the 3-amidation analogs **2c-i.** It was worth noting that the 3-esterification products **2a-b** were obtained as the side-products of the Negishi coupling of 6-iodide quinolone-3-ester **6b** with the zinc benzyl bromide and zinc 4-fluorobenzyl bromide, respectively.

Scheme 2. Synthesis of substituted 4-quinolone-3-carboxylic acid derivatives[a]

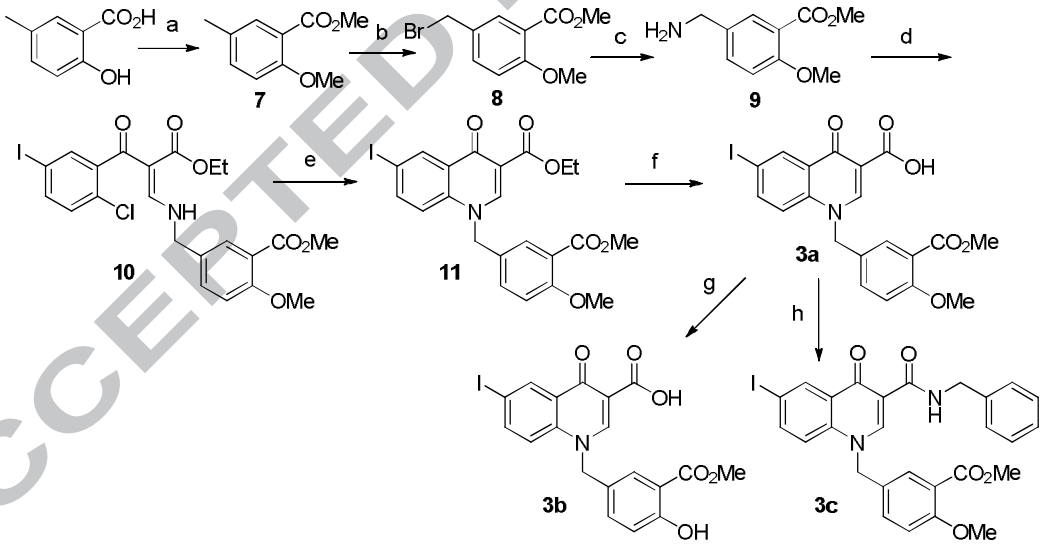


[a]Reagents and conditions: (a) $SOCl_2$, reflux; (b) ethyl 3-(dimethylamino)acrylate, Et_3N, toluene, reflux; (c) cyclopropylamine or benzylamine, Et_2O/EtOH (1/3, v/v), rt; (d) K_2CO_3, DMF, 100°C; (e) 6N HCl, MeOH, reflux; (f) i. Zinc dust, benzyl bromide or 4-fluorobenzyl iodide, TMSCl, $BrCH_2CH_2Br$, THF, 65°C, 2.5h; ii. $Pd_2(dba)_3$, tri(2-furyl)phosphine, THF, 65°C; (g) H_2, Pd/C (5%), MeOH, rt; (h) piperazine, NMP, 200°C, 0.5 hr, microwave irradiation; (i) aldehyde, $NaBH(OAc)_3$,

AcOH, DCE, rt, 6 h; (j) amine, BOP, Et_3N, DCM, rt (compounds **2a** and **2b** were isolated during the preparation of **6h** and **6i** under Negishi coupling reaction conditions).

Three classes of quinolone-3-carboxylic acid based bidentate inhibitors were designed with N-1, C-3 and C-6 as the attaching points using salicylic acid and aryl diketoacid as the second binding groups (**3a**-**h**). As shown in **Scheme 3**, the synthesis of N1-salicylic acid conjugated quinolone-3-carboxylates was commenced with 5-methylsalicylic acid, which was converted to the amino component **9** through bromination and amination. Then the nucleophilic attack of the enaminoester **4b** by the aminomethyl substituted salicylic ester **9** gave the quinolone-salicylic conjugate precursor **10**. The subsequent cyclization and hydrolysis/amidation furnished the target compounds **3a-c**.

Scheme 3. Synthetic route toward the N1-salicylic acid conjugated quinolone PTP1B inhibitors[a]

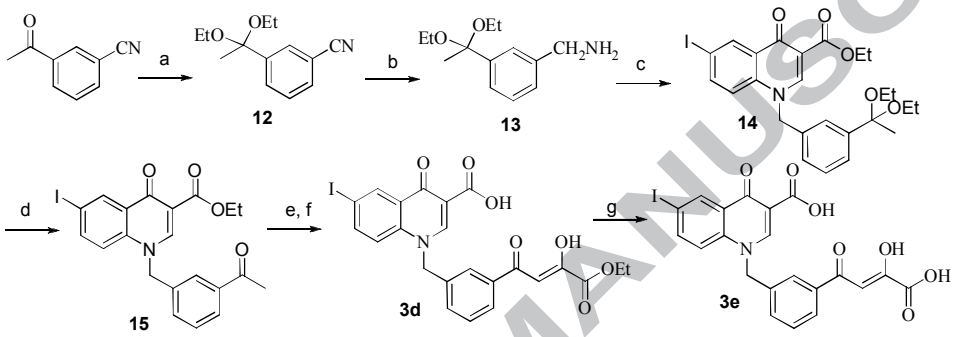


[a]Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, reflux; (b) Perbenzoic acid, NBS, CCl_4, reflux; (c) NH_3 (7 N in MeOH), 70°C, 2 hr; (d) **4e**, Et_2O / EtOH (1 / 3, v / v), rt; (e) K_2CO_3, DMF, 100°C; (f) 6N HCl, MeOH, reflux; (g) BBr_3, DCM, rt; (h) Benzylamine, Bop, Et_3N, DCM, rt.

Similarly, the attachment of an aryl diketoacid moiety on the N-1 site was

realized by the introduction of an aminomethyl substituted acetophenone, which was further transformed into the diketoacid functionality (**3d-e**). As described in Scheme 4, 3-acetylbenzonitrile served as the starting material and underwent the ketal protection and cyano reduction reactions, affording the amino component (**13**) for the N-1 substitution of the enaminoester **4e**.

Scheme 4. Synthetic route toward the N1-aryl diketoacid conjugated quinolone-3-carboxylate PTP1B inhibitors[a]

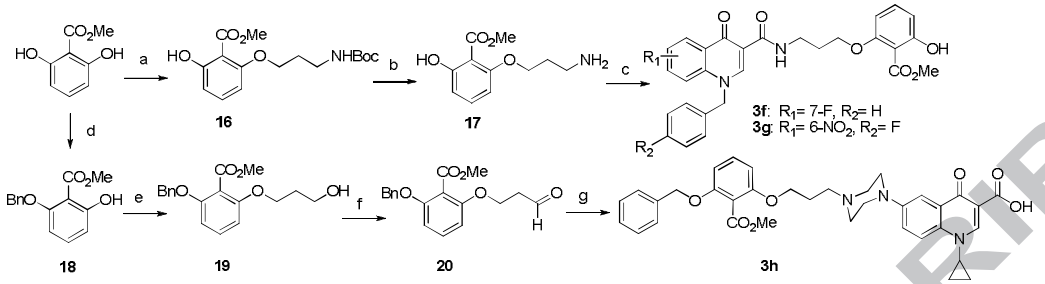


[a]Reagents and conditions: (a) p-toluenesulfonic acid, triethoxy methane, EtOH, rt; (b) Lithium aluminum hydride, anhydrous THF, 0°C -rt; (c) i. **4b**, Et_2O/EtOH (1/3, v/v), rt; ii. K_2CO_3, DMF, 100°C; (d) formic acid, rt; (e) 6N HCl, MeOH, reflux; (f) $(EtOOC)_2$, NaOMe, anhydrous THF, 50°C, 30 min; (g) 1N NaOH, 1N HCl, THF / EtOH (1 / 1, v / v), rt.

The C-3 conjugated bifunctional molecules (**3f-g**) were synthesized through a 3-oxypropylamino linker (Scheme 5). Starting from commercially available methyl 2,6-dihydroxybenzoate, etherification with 3-bromopropyl carbamate provided the alkylamino substituted salicylate (**16**). Then, amidation of various quinolone-3-carboxylic acids with the salicylic acid containing propylamine (**17**) furnished the C3-salicylate conjugated quinolone-3-carboxylic acid derivatives (**3f-g**).

We also tried the incorporation of the salicylate moiety into the C-6 position of the quinolone. An aldehyde group was introduced into the salicylate moiety as a handle (**20**) to join the quinolone motif by a reductive amination reaction, producing a C6-piperazino substituted quinolone-3-carboxylic acid (**3h**).

Scheme 5. Synthetic route toward the C-3/6 salicylic acid conjugated quinolone PTP1B inhibitors[a]



[a]Reagents and conditions: (a) *tert*-Butyl (3-bromopropyl)carbamate, K_2CO_3, DMF, 80°C; (b) TFA, DCM, rt; (c) **1g** or **1e**, Bop, Et_3N, DCM, rt; (d) benzyl bromide, K_2CO_3, anhydrous CH_3CN, 60°C; (e) 3-bromopropan-1-ol, K_2CO_3, anhydrous CH_3CN, 80°C; (f) Dess-Martin periodinane, anhydrous DCM, rt;(g) 1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, $NaBH(OAc)_3$, AcOH, DCE, rt.

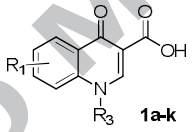
Results and Discussion

4-Quinolone-3-carboxylates with optimal hydrophobic substitution displayed effective inhibition against PTP1B enzyme. This is the first attempt to develop the 4-quinolone-3-carboxylic acid motif as an isostere of aryl diketoacid and as a new phosphotyrosine surrogate to improve cell permeability and PTP1B inhibition.

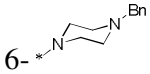
Initially, substitution with respect to the electronic and hydrophobic effect on the phenyl ring and N1-position were investigated. Typical substituents such as halogen, nitro, amino and phenyl group on the C6/7 position in combination with a hydrophobic group on N-1 site were assessed (**Table 1**). Several new quinolone-3-carboxylate compounds exhibited effective PTP1B inhibition. As expected, the introduction of a hydrophobic group into the quinolone-3-carboxylate motif was beneficial for the PTP1B binding. An optimal combination of a bulky benzyl group on the phenyl ring and a small cyclopropyl group on the N-1 position resulted in low micromolar inhibitory activity against the PTP1B enzyme (Table 1, **1h**, **1i**, **1k**). Notably, the planar hydrophobic group installed on the phenyl ring was superior to the N-1 position, as N1-benzyl substituted analogs displayed weak PTP1B

inhibition regardless of the substituents on the phenyl ring (Table 1, **1c-g**). However, double benzyl substitutions on C-6 and N-1 positions attenuated the inhibitory activity (**1j**). More significantly, the introduction of a hydrophobic group such as benzyl or 4-fluorobenzyl into the 3-carboxylic acid site by amidation or esterification generated neutral potent PTP1B inhibitors (**Table 2**, **2a-b**, **2g**), which might confer better cell permeability because of decreased charge. Interestingly, the C3-benzylamino substituent greatly boosted the PTP1B inhibitory activity of the inert N1-benzyl substituted quinolone (**2g** vs **1d**). This suggests a suitable combination of the hydrophobic groups on the polar 4-quinolone-3-carboxylic acid motif advances the PTP1B binding. As shown in Tables 1 and 2, an optimal combination includes an aromatic group at C-6/C-3 and a small alkyl group on N-1, or double aromatic substitutions at N-1/C-6 and C-3.

Table 1. Investigation on the phenyl substitution and the N1-substitution[a]

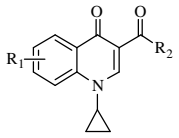


1a-k

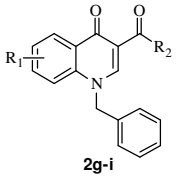
Compd.	R_1	R_3	PTP1B inhibition	
			% at 20 μg/mL	IC_{50} (μg/mL)[b]
1a	6-F	*c*-Pr	0.8	-
1b	6-I	*c*-Pr	3.5	-
1c	6-F	Bn	15.1	-
1d	6-I	Bn	3.1	-
1e	6-NO_2	4-F-Bn	36.2	-
1f	6-NH_2	4-F-Bn	12.4	-
1g	7-F	Bn	0.7	-
1h	6-Bn	*c*-Pr	87.5	7.5±1.0
1i	6-(4-F-Bn)	*c*-Pr	71.1	12.3±0.9
1j	6-Bn	Bn	15.1	-
1k	6- 	*c*-Pr	62.3	9.2±1.2

[a]Assay details were described in the Experimental section. [b] The pNPP competitive assay. IC_{50} values were determined by regression analysis and expressed as means ± SD of three independent replications.

Table 2. Investigation on the phenyl substitution and the carboxyl-derivatization[a]



2a-f



2g-i

Compd.	R_1	R_2	PTP1B inhibition	
			% at 20 μg/mL	IC_{50} (μg/mL)[b]
2a	6-I	OBn	80.5	11.4±1.7
2b	6-(4-F-Bn)	O-(4-F-Bn)	73.0	14.0±2.8
2c	6-I	NHBn	6.1	-
2d	6-I	NH-(4-F-Bn)	3.3	-
2e	6-F	NHBn	0.2	-
2f	7-F	NHBn	7.4	-
2g	6-I	NHBn	61.8	13.9±2.5
2h	6-F	NHBn	41.4	-
2i	6-Bn	OBn	0.1	-

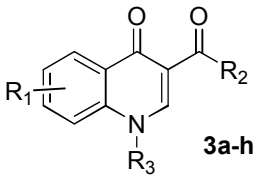
[a]Assay details were described in the Experimental section. [b] The pNPP competitive assay. IC_{50} values were determined by regression analysis and expressed as means ± SD of three independent replications.

Optimal conjugation of the quinolone-3-carboxylate with aryl diketoacid/salicylic acid enhanced the PTP1B inhibitory activity. We showed that the 4-quinolone-3-carboxylic acid, with suitable modifications, can serve as a new motif for effective PTP1B inhibition. Further structural optimization was turned to the bidentate ligand exploration to gain an improved PTP1B potency and selectivity over other PTPs. Taken into account our preliminary SAR study and synthetic accessibility, we chose 6-iodo-4-quinolone-3-carboxylic acid as a template to join the second binding moiety (Table 2, compounds **2a** and **2g**). In order to achieve an optimal

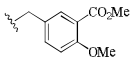
conjugation of the two functional motifs, we designed three types of bidentate inhibitors by employing the aryl diketoacid and salicylic acid as the second binding groups and incorporated them into the quinolone scaffold at the N-1, C-3 and C-6 positions, respectively (Table 3).

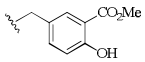
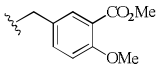
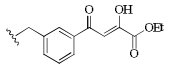
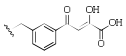
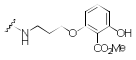
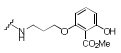
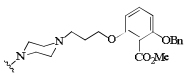
Most of these bidentate inhibitors exhibited improved PTP1B inhibitory activity compared to the corresponding quinolone-3-carboxylic acid counterpart. As shown in Table 3, the incorporation of a salicylic acid into the quinolone-3-carboxylic scaffold facilitated the enzyme inhibition (**3b vs 1d, 3f** vs. **1l**, **3g** vs **1j**), resulting in potent PTP1B inhibition with low micromolar IC_{50} values (Table 3, **3b**, IC_{50} = 8.1 μg/mL; **3f**, IC_{50} = 9.4 μg/mL; **3g**, IC_{50} = 6.5 μg/mL). As a comparison, the low potency of the methoxyl-substituted analogs **3a** and **3c** suggested that the free hydroxyl group of the salicylic acid moiety was critical for the PTP1B inhibitory activity. For the aryl diketoacid as the second binding group, its conjugate with the quinolone motif at N-1 position afforded the most potent PTP1B inhibitors among the quinolone series herein (Table 3, **3d**, IC_{50} = 3.12 μg/mL; **3e**, IC_{50} = 2.40 μg/mL). Our previous study showed that only the free aryl diketo acid exhibited PTP1B inhibitory activity and no effect was observed with the ester form. However, compound **3d** in which the aryl diketo acid was esterified still displayed a comparable activity with **3e**, probably due to the presence of 4-quinolone-3-carboxylic acid, which might function in a different inhibitory mechanism.

Table 3. The PTP1B inhibitory activity of the designed bidentate PTP1B inhibitors bearing the quinolone core structure[a]



3a-h

Compd.	R_1	R_2	R_3	IC_{50} (μg/mL) [b]
3a	6-I	OH		> 20

3b	6-I	OH		8.1±0.7
3c	6-I	NHBn		> 20
3d	6-I	OH		3.1±0.2
3e	6-I	OH		2.4±0.2
3f	7-F		Bn	9.4±1.5
3g	6-NO_2		4-F-Bn	6.5±1.4
3h	6- 	OH	*c*-Pr	45.2±8.5

[a]Assay details were described in the Experimental section. [b] The pNPP competitive assay. IC_{50} values were determined by regression analysis and expressed as means ± SD of three independent replications.

Kinetic analysis indicated that 4-quinolone-3-carboxylate acted as a competitive PTP1B inhibitor. The effect of the inhibitor on the PTP1B catalyzed reaction was studied with the substrate *p*-nitrophenyl phosphate (pNPP) in the presence and absence of different concentrations of **3d**. The kinetic parameters are summarized in Table 4. As the concentration of the inhibitor increased, the Km value of PTP1B increased accordingly while the Vmax value kept constant, demonstrating a competitive inhibition pattern with a Ki value of 1.33 μM.

Table 4. The kinetic parameters of PTP1B enzyme in the pNPP hydrolysis in the presence of different concentrations of the inhibitor **3d**[a].

[**Inhibitor**] (μM)	Km (mM)	Vmax (mOD/Min)
0.00	1.21	72.82
2.00	2.62	72.71
4.00	3.58	72.69
6.00	5.43	71.64

8.00	7.40	68.65

[a] Km is the Michaelis constant, Vmax is the maximum rate and were calculated from the Michaelis-Menten equation.[28]

Further Lineweaver-Burk plot, as shown in **Figure 1**, confirmed the competitive inhibition mechanism by the quinolone-4-carboxylate based PTP1B inhibitors. This mode of inhibition was contrary to that of the aryl diketoacid-based PTP1B inhibitors, which were active site-directed non-competitive inhibitors by targeting an inactive conformation of the enzyme.[21]

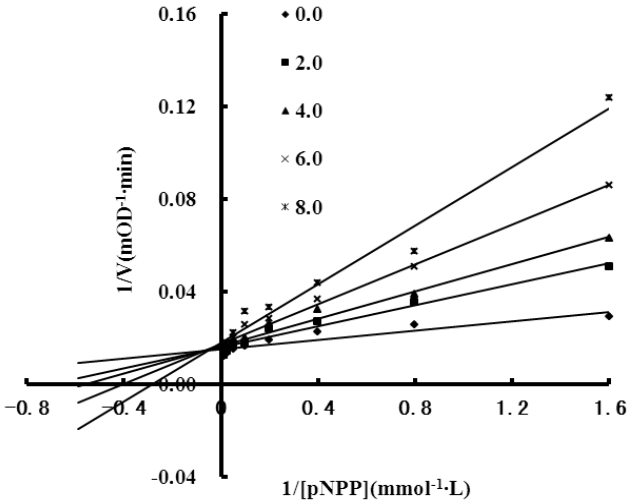


Figure 1. The effect of **3d** on PTP1B-catalyzed pNPP hydrolysis. The experiment was performed at 25 °C and pH 7.0. The inhibitor **3d** concentrations were 0 (◆), 2.0 (■), 4.0 (▲), 6.0 (×) and 8.0 (∗) μM, respectively.

The quinolone-3-carboxylate displayed selective inhibition against PTP1B. Several active compounds with representative structures (**1c**, **1d, 2g**, **3b**, **3d**, **3e** and **3g**) were selected to test against a panel of PTPs including cytosolic PTPs, TCPTP, the closest homologue of PTP1B (74% sequence identity in their catalytic domains),[29] SHP1, SHP2 and the receptor-like PTPs, LAR. As shown in Table 5, all these inhibitors demonstrated a moderate to excellent PTP1B selectivity over other PTPs. For monomer quinolones **1c**, **1d** and **2g**, no inhibition against other PTPs was

observed at 20 μg/mL, indicating that 4-quinolone-3-carboxylic acid motif was selective for PTP1B. The bidentate inhibitors **3b** and **3g** bearing salicylic acid moiety exhibited a better potency and higher selectivity for PTP1B. The most potent aryl diketoacid-quinolone conjugates **3d** and **3e** displayed a selective inhibition of PTP1B over TCPTP by a factor of 2-fold, and at least 10-fold over SHP2 and LAR.

Table 5. The inhibitory activity of select quinolone-based PTP1b inhibitors against a panel of PTPs, IC_{50} (μg/mL) [a]

Compd.	PTP1B	TCPTP	SHP1	SHP2	LAR
1h	7.5±1.0	NA[b]	NA	NA	NA
1i	12.3±0.9	NA	NA	NA	NA
2g	13.9±2.5	NA	NA	NA	NA
3b	8.1±0.7	NA	NA	NA	NA
3d	3.1±0.2	6.3±0.7	6.8±1.1	NA	NA
3e	2.4±0.2	4.9±0.7	5.1±0.4	NA	NA
3g	6.5±1.34	NA	NA	NA	NA

[a] The pNPP assay. IC_{50} values were determined by regression analysis and expressed as means ± SD of three replications. [b] Inhibition rate < 50% at 20 μg/ mL concentration.

The quinolone-3-carboxylate exhibited distinct cellular efficacy. Previous studies have illustrated that PTP1B negatively regulates the insulin activated signaling pathways by catalyzing the dephosphorylation of the insulin receptor β (IRβ) subunit. Thus, inhibition of PTP1B activity should enhance insulin signaling. To determine the cellular efficacy of **3d**, we assessed its effect on insulin signaling in CHO/*h*IR cells. As shown in Figure 2, **3d** markedly increased insulin stimulated IRβ phosphorylation at 90 μM concentration while there was no significant effect at the lower concentrations.

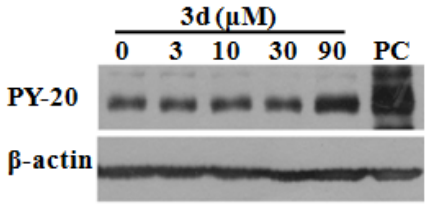
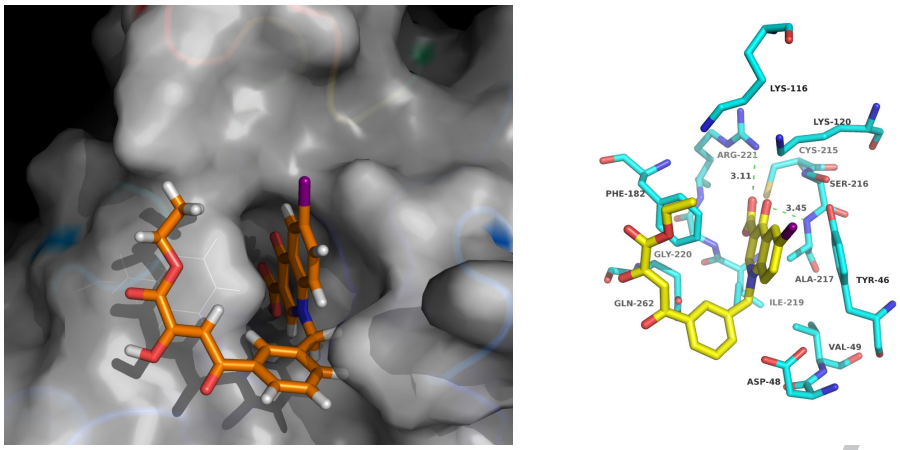


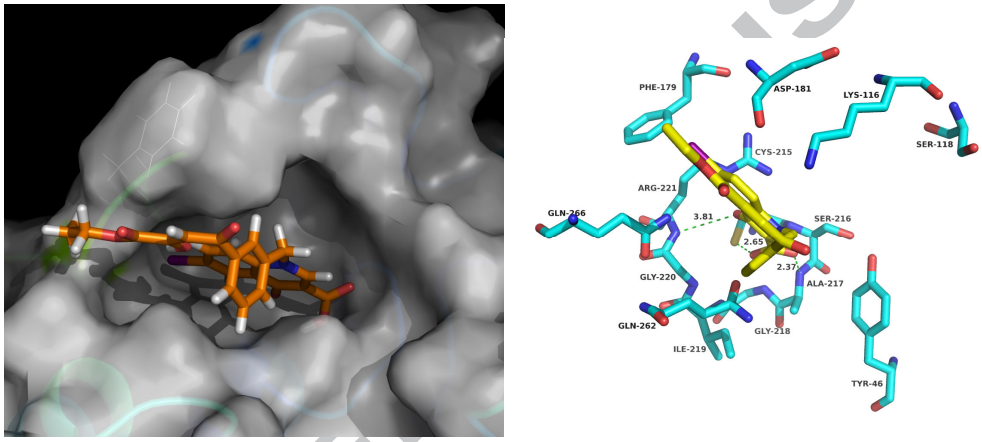
Figure 2. Effect of **3d** on insulin-mediated IRβ phosphorylation in CHO/*h*IR cells.

The quinolone-3-carboxylates bind to PTP1B in the active site interacting with the WPD-loop closed conformation, as predicted by molecular modeling. Finally, we tried to interpret the binding mode of the quinolone-3-carboxylate motif by the molecular modeling. Based on the X-ray cocrystal structure of the PTP1B inhibitor bound to the active site of the enzyme in the WPD loop open conformation (PDB: 3QKP),[30] and in WPD loop closed conformation (PDB: 1PXH),[31] we predicted the binding mode of the lead inhibitor **3d** in the PTP1B domain using Autodock (Autodock.Scripps.edu).

When docking **3d** into the active pocket of the enzyme in both conformations, we observed that this compound binding to WPD loop closed conformation was more stable (Figure 3A). The quinolone ring resided well to position the carboxyl oxygen in hydrogen bonding distance to Ala217, Arg221, Ile219 and Gly220 residues, and the phenyl ring of the quinolone formed a π-π stacking interaction with the Try46 residue. However, when ligand **3d** binding to WPD loop open conformation (Figure 3B), the carboxyl oxygen of quinolone only formed two hydrogen bonds with Ala217 and Arg221 residues, and no π-π stacking interaction existed. Further calculation of the binding free energy of two ligand-protein complexes confirmed that this compound preferred binding the WPD-closed conformation to the WPD-open conformation, i.e. ΔG-WPD-open (-43.623kcal/mol) > ΔG-WPD-close (-68.453kcal/mol). The binding mode established by the molecular modeling was consistent with the kinetic experiment of **3d** showing a competitive PTP1B inhibition.



A) WPD loop closed, ΔG = -68.45 kcal/mol



B) WPD loop open, ΔG = -43.623kcal/mol

Figure 3. A proposed binding mode of **3d** to the PTP1B enzyme, based on the X-ray co-crystal structure of PTP1B in complex with a small molecule inhibitor in WPD loop closed (**3A**)[31] or WPD loop open state (**3B**).[30]

Conclusions

By employing bioisostere replacement strategy, we introduced 4-quinolone-3-carboxylic acid motif into the aryl diketoacid chemotype for the design and discovery of novel PTP1B inhibitors. Efficient synthesis was established to provide variously substituted quinolone-3-carboxylic acid derivatives for an optimal template exploration. The SAR study revealed that hydrophobic group was favored at C6-position of the quinolone ring and the steric effect at N1-position interfered with the binding. By introducing salicylic acid or aryl diketoacid as a second binding group

into the quinolone motif, several bidentate inhibitors were obtained with improved inhibitory activity and good selectivity. The C3-aryl diketoacid conjugated quinolones (**3d** and **3e**) displayed the highest PTP1B inhibitory activity with low micromolar IC_{50} values, acting as a competitive inhibitor. More significantly, **3d** possesses distinct cellular efficacy in inhibiting IRβ phosphorylation in CHO/*h*IR cells. In summary, we identified for the first time the 4-quinolone-3-carboxylic acid as a novel surrogate of phosphotyrosine leading to the design of potent PTP1B inhibitors with excellent selectivity and cell permeability.

Experimental Section

General Synthetic Methods.

All Reagents and solvents were obtained from commercial sources in analytical grade and used without further purification. Melting points were determined by SGW X-4B; ^{1}H NMR spectra were recorded on a Varian Mercury-400 or 300 MHz and ^{13}C NMR spectra were recorded on a Varian Mercury-125 MHz. Mass specta were recorded using a Finnigan MAT-95 instrument for EI and Finnigan LCQ Deca for ESI.

General procedure for the preparation of the key intermediate **6**: the acid chlorides of commercially available substituted benzoic acids were coupled with ethyl 3-(dimethylamino)acrylate to produce the acrylates **4**. Substitution with cyclopropylamine or benzylamine and subsequent cyclization with potassium carbonate gave quinolone-3-carboxylate **6**.

Ethyl 2-(2,5-difluorobenzoyl)-3-(dimethylamino)acrylate (4a). A mixture of 2,5-difluorobenzoic acid (1.58 g, 10 mmol) and thionyl chloride (7 mL) was refluxed for 3 hrs. The excess thionyl chloride was removed by distillation under reduced pressure to give an oil residue which was then dissolved in dry toluene (25 mL). To this solution was added the mixture of (*E*)-ethyl 3-(dimethylamino)acrylate (1.43 mL, 10 mmol) and dry Et_3N (2.1 mL, 10 mmol). The resulting solution was heated at 90 °C for 2 hrs. After cooling and filtering, the solvent was evaporated to dryness and the residue was purified by silica gel column chromatography eluting with PE /

EtOAc (10 / 1) to give **4a** (2.83 g, 100%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 6.89 (td, *J* = 8.3, 2.4 Hz, 1H), 6.81 – 6.70 (m, 1H), 3.99 (q, *J* = 7.1 Hz, 2H), 3.28 (s, 3H), 2.91 (s, 3H), 0.95 (t, *J* = 7.1 Hz, 3H).

Ethyl 1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6a). To a stirred solution of **4a** (283 mg, 1 mmol) in EtOH (3 mL) and Et_2O (1 mL) was added cyclopropylamine (93 mg, 1.63mmol) dropwise. After stirring at room temperature for 15 min, the mixture was evaporated to dryness to give compound **5a** as a white solid, which was used directly for the next step. The crude product **5a** was dissolved in dry DMF (2 mL) and was added K_2CO_3 (248 mg, 1.8 mmol). The reaction mixture was heated at 100 °C for 40 min, then cooled to room temperature and poured into ice-water. The precipitate was filtered off, washed with water, and dried to give **6a** (248 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.49 (dd, *J* = 8.9, 3.0 Hz, 1H), 8.29 (dd, *J* = 9.3, 4.3 Hz, 1H), 7.18 (ddd, *J* = 9.3, 7.5, 3.0 Hz, 1H), 4.75 (q, *J* = 7.1 Hz, 2H), 3.88 – 3.79 (m, 1H), 1.77 (t, *J* = 7.1 Hz, 3H), 1.74 – 1.66 (m, 2H), 1.54 – 1.47 (m, 2H).

Ethyl 2-(2-chloro-5-iodobenzoyl)-3-(dimethylamino)acrylate (4b). Analogous to the preparation of **4a**, 2-chloro-5-iodobenzoic acid (2.81 g, 10 mmol) was treated with thionyl chloride (7 mL) followed by the addition of (*E*)-ethyl 3-(dimethylamino)acrylate (1.43 mL, 10 mmol) to give **4b** (4.0 g, 100%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.67 (s, 1H), 7.60 – 7.54 (m, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 3.92 (q, *J* = 7.1 Hz, 2H), 3.33 (s, 3H), 2.97 (s, 3H), 0.88 (t, *J* = 7.1 Hz, 3H).

Ethyl 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (6b). Analogous to the preparation of **6a**, **4b** (407 mg, 1.0 mmol) was treated with cyclopropylamine (93 mg, 1.63 mmol) followed by cyclization to afford 352 mg of **6b** (92%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.1 Hz, 1H), 8.59 (s, 1H), 7.96 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.44 (tt, *J* = 7.1, 3.9 Hz, 1H), 1.41 (t, *J* = 7.1 Hz, 3H), 1.36 – 1.30 (m, 2H), 1.15 – 1.10 (m, 2H).

Ethyl 1-benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6c). According to the same procedure described for **6a**, **4a** (283 mg, 1.0 mmol) was treated with benzylamine (0.18 mL, 1.63 mmol) followed by cyclization to afford 310 mg (95%) of **6c** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.21 – 8.16 (m, 1H), 7.42 – 7.32 (m, 3H), 7.32 – 7.27 (m, 2H), 7.18 – 7.12 (m, 2H), 5.40 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H).

Ethyl 1-benzyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (6d). According to the same procedure described for **6a**, **4b** (407 mg, 1.0 mmol) was treated with benzylamine (0.18 mL, 1.63mmol) followed by cyclization to afford 390 mg (90%) of **6d** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (d, *J* = 2.1 Hz, 1H), 8.59 (s, 1H), 7.79 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.41 – 7.32 (m, 3H), 7.14 (dd, *J* = 7.2, 2.2 Hz, 2H), 7.06 (d, *J* = 8.9 Hz, 1H), 5.36 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H).

Ethyl 1-(4-fluorobenzyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6e). 6e was prepared in a similar fashion as described for **6a**, yellow solid, 84%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 – 8.10 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.20 – 7.04 (m, 3H), 5.67 (s, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H).

Ethyl 1-benzyl-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6f). 6f was prepared in a similar fashion as described for **6a**, a white solid, 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.54 (dd, *J* = 9.0, 6.4 Hz, 1H), 7.42 – 7.33 (m, 3H), 7.17 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.13 – 7.07 (m, 1H), 7.00 – 6.95 (m, 1H), 5.34 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H).

Ethyl 6-benzyl-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (6g). Zinc powder (195 mg, 3.0 mmol) was placed in a three-neck flask. Anhydrous THF (1.0 mL) was added. Then TMSCl (4 μL, 0.03 mmol) and 1,2-dibromomethane (20 μL, 0.015 mmol) were added sequentially. The mixture was stirred at rt for 10 min. Benzyl bromide (0.18 mL, 1.5 mmol) was added slowly to keep the solvent gently refluxing. After addition, the mixture was stirred at 65°C for 2.5 hr. Compound **6b** (383 mg, 1.0 mmol), $Pd_2(dba)_3$ (23 mg, 0.04 mmol) and trifurylphosphine (19 mg,

0.08 mmol) were placed in a two-neck flask, evacuated and refilled with N_2. Then anhydrous THF (3.0 mL) was added. The solution was stirred at 65°C for reflux. The zinc bromide mixture was added dropwise to the solution, then stirred at this temperature for 3 hrs. Saturated NH_4Cl solution was added and extracted with ethyl acetate (20 mL). The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4.The solvent was evaporated to give a residue which was purified by SGC to give 240 mg of **6g** as a white solid (69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.38 (d, *J* = 2.6 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.32 – 7.26 (m, 2H), 7.23 – 7.17 (m, 3H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.11 (s, 2H), 3.48 – 3.39 (m, 1H), 1.41 (t, *J* = 7.1 Hz, 3H), 1.30 – 1.25 (m, 2H), 1.15 – 1.08 (m, 2H); ESI *m/z*: 348.1 $(M+H)^+$.

Ethyl 1-cyclopropyl-6-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (6h). According to the same procedure described for **6g**, **6b** (383 mg, 1.0 mmol) was treated with 4-fluorobenzyl zinc bromide (190 mg, 1.5 mmol), giving 157 mg (43%) of **6h** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.33 (d, *J* = 2.0 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.63 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.18 (dd, *J* = 10.4, 3.8 Hz, 2H), 7.03 – 6.92 (m, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 4.12 (s, 2H), 3.60 – 3.49 (m, 1H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.31 – 1.24 (m, 2H), 1.17 – 1.12 (m, 2H).

Ethyl 1,6-dibenzyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (6i). According to the same procedure described for **6g**, **6d** (433 mg, 1.0 mmol) was treated with benzyl zinc bromide (0.18 mL, 1.5 mmol) to afford 203 mg (51%) of **6i** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.41 (d, *J* = 2.1 Hz, 1H), 7.41 – 7.28 (m, 5H), 7.26 – 7.23 (m, 2H), 7.22 – 7.19 (m, 1H), 7.16 (ddd, *J* = 9.5, 5.8, 1.8 Hz, 4H), 5.36 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 4.05 (s, 2H), 1.42 (t, *J* = 7.1 Hz, 3H).

1-Cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1a). To the solution of **6a** (230 mg, 0.88 mmol) in MeOH (3.0 mL) was added HCl (6N, 3.0 mL). The solution was stirred at reflux for 2 hr. The solvent was evaporated to give a white solid. Then the solid was washed with 20 mL EtOH and dried in the oven, affording **1a** as a white solid (187 mg, 86%). Mp: 272-273°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.62 (s, 1H), 8.88 (s, 1H), 8.15 – 8.12 (m, 2H), 7.64 – 7.57 (m, 1H), 3.68 – 3.58 (m,

1H), 1.48 – 1.39 (m, 2H), 1.27 – 1.22 (m, 2H); EI-MS *m/z*: 247 (M^+); HRMS (EI) (M^+): calcd for $C_{13}H_{10}FNO_3$ 247.0661, found 247.0653.

1-Cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1b). According to the same procedure described for **1a**, **6b** (188 mg, 0.49 mmol) was treated with HCl (6N, 3.0 mL) to afford 151 mg (85%) of **1b** as a white solid. Mp: 243-245°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.85 – 8.84 (m, 1H), 8.13 – 8.09 (m, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 3.62 – 3.56 (m, 1H), 1.46 – 1.39 (m, 2H), 1.26 – 1.17 (m, 2H); ESI-MS *m/z*: 355.98 $(M+H)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{13}H_{11}O_3NI$ 355.9778, found 355.9789.

1-Benzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1c). According to the same procedure described for **1a**, **6c** (98 mg, 0.3 mmol) was treated with HCl (6N, 1.2 mL) to afford 71 mg (80%) of **1c** as a white solid. Mp: 237-239°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.20 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.57 – 7.37 (m, 5H), 7.15 (dd, *J* = 7.3, 2.3 Hz, 2H), 5.52 (s, 2H); ESI-MS *m/z*: 320.07 $(M+Na)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{17}H_{12}O_3NFNa$ 320.0693, found 320.0702.

1-Benzyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1d). According to the same procedure described for **1a**, **6d** (150 mg, 0.35 mmol) was treated with HCl (6N, 2.0 mL) to afford 126 mg (90%) of **1d** as a white solid. Mp: 207-209°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.86 (s, 1H), 9.30 (s, 1H), 8.64 (d, *J* = 2.0 Hz, 1H), 8.15 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.65 (d, *J* = 9.1 Hz, 1H), 7.40 – 7.22 (m, 5H), 5.86 (s, 2H); ESI-MS *m/z*: 405.99 $(M+H)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{17}H_{13}O_3NI$ 405.9935, found 405.9933.

1-(4-Fluorobenzyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1e). According to the same procedure described for **1a**, **6e** (200 mg, 0.54 mmol) was treated with HCl (6N, 4.0 mL) to afford 124 mg (67%) of **1e** as a light yellow solid. Mp: 227-228°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 9.01 (d, *J* = 2.0 Hz, 1H), 8.58 (d, *J* = 9.3 Hz, 1H), 8.07 (d, *J* = 9.2 Hz, 1H), 7.41 – 7.38 (m, 2H), 7.20 (t, *J* = 8.6 Hz, 2H), 5.91 (s, 2H); ESI-MS *m/z*: 343.07 $(M+H)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{17}H_{12}O_5N_2F$ 343.0725, found 343.0725.

6-Amino-1-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1f).

The reaction mixture of **1e** (162 mg, 0.5 mmol) and Pd/C (5%, 8 mg) in anhydrous MeOH was stirred under the atmosphere of H_2 for 6 hr at rt. The solution was filtered through a pad of Celite and the filtrate was collected. The solvent was evaporated to give a crude product which was purified by Prep-HPLC, affording 118 mg (80%) of **1f** as a yellow solid. Mp: 261-263°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.36 (d, *J* = 2.6 Hz, 1H), 8.98 (s, 1H), 8.50 (dd, *J* = 9.3, 2.5 Hz, 1H), 7.66 (d, *J* = 9.7 Hz, 1H), 7.24 – 7.05 (m, 4H), 5.57 (s, 2H); EI-MS *m/z*: found 312 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{17}H_{13}FN_2O_3$ 312.0983, found 312.0991.

Benzyl-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1g). According to the same procedure described for **1a**, **6f** (100 mg, 0.31 mmol) was treated with HCl (6N, 1.2 mL) to afford 87 mg (95%) of **1g** as a white solid. Mp: 227-230°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (s, 1H), 8.92 (s, 1H), 8.55 – 8.46 (m, 1H), 7.36 (d, *J* = 6.8 Hz, 3H), 7.16 (s, 3H), 7.05 (d, *J* = 8.2 Hz, 1H), 5.40 (s, 2H), 3.05 – 2.93 (m, 1H), 0.90 – 0.80 (m, 2H), 0.71 – 0.63 (m, 2H); EI-MS *m/z*: 297 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{13}H_{12}FNO_3$ 297.0802, found 297.0794.

6-Benzyl-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1h). According to the same procedure described for**1a**, **6g** (70 mg, 0.2 mmol) was treated with HCl (6N, 1.0 mL) to afford 58 mg (90%) of **1h** as a white solid. Mp: 193-195°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.35 – 7.28 (m, 2H), 7.26 – 7.18 (m, 3H), 4.16 (s, 2H), 3.64 – 3.52 (m, 1H), 1.43 – 1.34 (m, 2H), 1.19 (dt, *J* = 11.0, 5.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.6, 167.1, 147.8, 140.1, 139.8, 139.6, 135.0, 128.9, 128.9, 126.6, 126.3, 126.0, 117.5, 108.6, 41.4, 35.4, 8.2; EI-MS *m/z*: 319 (M^+); HRMS (EI) (M^+) calcd for $C_{20}H_{17}NO_3$ 319.1208, found 319.1207.

Cyclopropyl-6-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1i). According to the same procedure described for **1a**, **6h** (53 mg, 0.15 mmol) was treated with HCl (6N, 1.0 mL) to afford 46 mg of **1i** (92%) as a white solid. Mp: 213-216°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.90 (s, 1H), 8.86 (s, 1H), 8.35 (d, *J* = 2.0 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.64 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.16 (dd, *J* = 10.4, 3.8 Hz, 2H), 7.04 – 6.95 (m, 2H), 4.13 (s, 2H), 3.63 – 3.53 (m, 1H), 1.39 (q, *J* = 6.5

Hz, 2H), 1.22 – 1.18 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.5, 167.0, 161.6 (d, *J* = 245.1 Hz), 147.8, 139.9, 139.6, 135.4, 134.8, 130.4 (*J* = 7.5 Hz), 126.2, 126.0, 117.6, 115.6 (d, *J* = 21.3 Hz), 108.7, 40.6, 35.3, 8.2; EI-MS *m/z*: 337 (M^+); HRMS (EI) (M^+) calcd for $C_{20}H_{16}NO_3F$ 337.1114, found 337.1111.

1,6-Dibenzyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1j). According to the same procedure described for **1a**, **6i** (102 mg, 0.26 mmol) was treated with HCl (6N, 1.5 mL) to afford 81 mg of **1j** (85%) as a white solid. Mp: 199-201°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 8.87 (s, 1H), 8.41 (d, *J* = 1.8 Hz, 1H), 7.51 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 7.40 – 7.32 (m, 3H), 7.32 – 7.27 (m, 2H), 7.24 – 7.07 (m, 5H), 5.48 (s, 2H), 4.11 (s, 2H); ESI-MS *m/z*: 370.2 $(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{24}H_{19}NO_3Na$ 392.1263, found 392.1255.

6-(4-Benzylpiperazin-1-yl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1k). A solution of compound **1a** (247 mg, 1.0 mmol) and piperazine (258 mg, 3.0 mmol) in NMP (0.5 mL) was stirred under microwave heating at 200°C for 0.5 hr, then cooled to rt. After evaporating the solvent, the residue was diluted with EtOH and filtered, washed with EtOH, giving a yellow solid which was used directly in the next step. To the solution of the solid obtained above (313 mg, 1.0 mmol) in DCE (5.0 mL) were added AcOH (86 μL, 1.5 mmol), $NaBH(OAc)_3$ (509 mg, 2.4 mmol) and benzaldehyde (0.15 mL, 1.5 mmol) at rt. The mixture was stirred for 6 hr at rt, then diluted with DCM (20 mL). Washed with saturated $NaHCO_3$ (5.0 mL) solution, brine and dried over anhydrous Na_2SO_4. Concentrated, and the residue was purified by SGC to give 174 mg (43%) of **1k** as a light yellow solid. Mp: 223-225°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.24 (s, 1H), 8.73 (s, 1H), 7.97 (d, *J* = 9.4 Hz, 1H), 7.76 (d, *J* = 2.9 Hz, 1H), 7.46 (dd, *J* = 9.4, 2.9 Hz, 1H), 7.35 (d, *J* = 2.0 Hz, 2H), 7.30 (dd, *J* = 5.8, 2.9 Hz, 1H), 7.19 (t, *J* = 7.9 Hz, 2H), 6.86 (dd, *J* = 7.9, 4.9 Hz, 2H), 3.60 – 3.55 (m, 1H), 3.37 (d, *J* = 2.4 Hz, 4H), 2.85 (s, 2H), 2.70 – 2.62 (m, 4H), 1.35 (q, *J* = 6.8 Hz, 2H), 1.21 – 1.14 (m, 2H); EI-MS *m/z*: 403 (M^+); HRMS (EI) $(M)^+$ calcd for $C_{24}H_{25}N_3O_3$ 403.1896, found 403.1890.

Benzyl 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (2a). According to the same procedure described for **6g**, **6b** (376 mg, 1.0 mmol) was

treated with benzyl zinc bromide (0.18 mL, 1.5 mmol), producing 25 mg (5.6%) of **2a** as a white solid. Mp: 154-156°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.1 Hz, 1H), 8.59 (s, 1H), 7.96 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.42 – 7.29 (m, 3H), 5.39 (s, 2H), 3.43 (ddd, *J* = 10.7, 7.2, 3.9 Hz, 1H), 1.35 – 1.27 (m, 2H), 1.11 (qd, *J* = 5.7, 2.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 165.0, 148.9, 141.0, 140.0, 136.3, 129.7, 128.6, 128.0, 128.0, 123.5, 118.6, 111.0, 90.1, 66.4, 34.6, 8.2; ESI-MS *m/z*: 468.2 $(M+Na)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{20}H_{16}NO_3I$ 468.0073, found 468.0084.

4-Fluorobenzyl-1-cyclopropyl-6-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (2b). According to the same procedure described for **6g**, **6b** (383 mg, 1.0 mmol) was treated with 4-fluorobenzyl zinc bromide (190 mg, 1.5 mmol) to afford 149 mg (32%) of **2b** as a white solid. Mp: 136-138°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.39 – 8.35 (m, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.53 – 7.49 (m, 3H), 7.18 – 7.12 (m, 2H), 7.05 (t, *J* = 8.8 Hz, 2H), 6.96 (t, *J* = 8.9 Hz, 2H), 5.36 (s, 2H), 4.07 (s, 2H), 3.48 – 3.40 (m, 1H), 1.30 (d, *J* = 9.0 Hz, 2H), 1.14 – 1.08 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 165.8, 162.5 (d, *J* = 243.8 Hz), 161.5 (d, *J* = 240.0 Hz), 148.5, 139.0, 138.6, 136.0, 133.3, 132.2, 130.3 (d, *J* = 7.9 Hz), 129.8 (d, *J* = 8.2 Hz), 128.6, 127.2, 116.9, 115.5 (d, *J* = 5.0 Hz), 115.3 (d, *J* = 5.0 Hz), 110.5, 65.9, 40.6, 34.5, 8.1; ESI-MS *m/z*: 446.3 $(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{27}H_{21}NO_3NaF_2$ 468.1387, found 468.1381.

Benzyl 1,6-dibenzyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (2i). According to the same procedure described for **6g**, **6d** (433 mg, 1.0 mmol) was treated with benzyl zinc bromide (0.18 mL, 1.5 mmol) to afford 106 mg (23%) of **2i** as a white solid. Mp: 147-149°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.43 (s, 1H), 7.55 – 7.50 (m, 2H), 7.42 – 7.29 (m, 7H), 7.24 (s, 2H), 7.22 – 7.10 (m, 6H), 5.41 (s, 2H), 5.34 (s, 2H), 4.05 (s, 2H); ESI-MS *m/z*: 460.4 $(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{31}H_{25}NO_3Na$ 482.1732, found 482.1722.

N-benzyl-1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxamide (2c). To a solution of **1b** (75 mg, 0.21 mmol) in DCM (2mL) was added Bop (111 mg, 0.25 mmol) and Et_3N (73 ul, 0.53 mmol) at rt, and the reaction mixture was stirred for 15

min. Then benzylamine (27 mg, 0.25 mmol) was added and the reaction mixture was stirred for 2 hrs. The reaction mixture was dissolved in DCM (15 mL) and washed with water. The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 74 mg (80%) of **2c** as a white solid. Mp: 169-171°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (t, *J* = 6.3 Hz, 1H), 8.93 (s, 1H), 8.79 (d, *J* = 2.0 Hz, 1H), 8.00 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.35 (dt, *J* = 15.0, 7.4 Hz, 4H), 7.23 (d, *J* = 7.2 Hz, 1H), 4.67 (d, *J* = 5.8 Hz, 2H), 3.54 – 3.45 (m, 1H), 1.39 – 1.30 (m, 2H), 1.20 – 1.12 (m, 2H); EI-MS *m*/*z*: 444 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{20}H_{17}IN_2O_2$ 444.0331, found 444.0329.

1-Cyclopropyl-N-(4-fluorobenzyl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxamide (2d). According to the same procedure described for **2c**, **1b** (107 mg, 0.3 mmol) was treated with 4-fluorobenzylamine (46 mg, 0.36 mmol) to afford 115 mg (83%) of **2d** as a white solid. Mp: 178-180°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (t, *J* = 6.1 Hz, 1H), 8.91 (s, 1H), 8.78 (d, *J* = 1.8 Hz, 1H), 8.00 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.33 (dd, *J* = 8.1, 5.6 Hz, 2H), 7.00 (t, *J* = 8.6 Hz, 2H), 4.62 (d, *J* = 5.7 Hz, 2H), 3.55 – 3.44 (m, 1H), 1.34 (q, *J* = 6.9 Hz, 2H), 1.21 – 1.11 (m, 2H); EI-MS *m*/*z*: 462 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{20}H_{16}F\ I\ N_2O_2$ 462.0257, found 462.0249.

***N*-Benzyl-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide (2e).** According to the same procedure described for **2c**, **1a** (25 mg, 0.1 mmol) was treated with benzylamine (13 mg, 0.12 mmol) to afford 30 mg (90%) of **2e** as a white solid. Mp: 161-163°C; ^{1}H NMR (300 MHz, CD_3OD) δ 10.55 (t, *J* = 7.4 Hz, 1H), 8.90 (s, 1H), 8.30 (dd, *J* = 9.4, 4.4 Hz, 1H), 8.01 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.67 (ddd, *J* = 9.4, 7.8, 3.1 Hz, 1H), 7.40 – 7.22 (m, 4H), 4.64 (d, *J* = 5.8 Hz, 2H), 3.79– 3.72 (m, 1H), 1.42 – 1.32 (m, 2H), 1.23 – 1.15 (m, 2H); ESI-MS *m*/*z*: 359.2 $(M+Na)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{20}H_{19}N_2O_2NaF$ 359.1171, found 359.117.

***N*-Benzyl-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide (2f).** According to the same procedure described for **2c**, **1g** (50 mg, 0.2 mmol) was treated with benzylamine (26 mg, 0.24 mmol) to afford 58 mg (87%) of **2f** as a white solid. Mp: 165-167°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.29 (t, *J* = 6.2 Hz, 1H), 8.93 (s, 1H),

8.49 (dd, *J* = 9.0, 6.3 Hz, 1H), 7.65 (dd, *J* = 10.3, 2.3 Hz, 1H), 7.41 – 7.30 (m, 4H), 7.24 – 7.18 (m, 1H), 4.67 (d, *J* = 5.8 Hz, 2H), 3.52 – 3.43 (m, 1H), 1.41 – 1.33 (m, 2H), 1.18 (dt, *J* = 3.9, 3.0 Hz, 2H); EI-MS *m/z*: 336 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{20}H_{17}F\ N_2O_2$ 336.1276, found 336.1275.

***N*,1-Dibenzyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxamide (2g).** According to the same procedure described for **2c**, **1d** (81 mg, 0.2 mmol) was treated with benzylamine (26 mg, 0.24 mmol) to afford 89 mg (90%) of **2g** as a white solid. Mp: 171-174°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.33 – 10.27 (m, 1H), 8.95 (s, 1H), 8.84 (d, *J* = 2.1 Hz, 1H), 7.84 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.43 – 7.27 (m, 8H), 7.18 – 7.12 (m, 3H), 5.44 (s, 2H), 4.70 (d, *J* = 5.8 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.4, 164.5, 148.8, 141.3, 138.6, 136.4, 133.8, 129.4, 128.8, 128.6, 127.6, 127.1, 125.0, 118.6, 112.7, 111.0, 100.9, 89.9, 57.8, 43.2; ESI-MS *m/z*: 337.1 $(M+H)^+$; HRMS (ESI) $(M+H)^+$ calcd for $C_{24}H_{19}N_2O_2I$ 495.0570, found 495.0549.

***N*,1-Dibenzyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide (2h).** According to the same procedure described for **2c**, **1c** (75 mg, 0.25 mmol) was treated with benzylamine (33 mg, 0.3 mmol) to afford 65 mg (63%) of **2h** as a white solid. Mp: 166-168°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.34 (t, *J* = 5.5 Hz, 1H), 8.97 (s, 1H), 8.15 (dd, *J* = 8.9, 2.9 Hz, 1H), 7.48 – 7.27 (m, 10H), 7.18 – 7.12 (m, 2H), 5.47 (s, 2H), 4.70 (d, *J* = 5.8 Hz, 2H); ESI-MS *m/z*: 397.2 $(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{24}H_{19}N_2O_2NaF$ 409.1328, found 409.1334.

Methyl 2-methoxy-5-methylbenzoate (7). To a solution of 2-hydroxy-5-methylbenzoic acid (761 mg, 5 mmol) and K_2CO_3 (2.07 g, 15 mmol) in anhydrous acetone (15 mL) was added CH_3I (0.78 mL, 12.5 mmol) dropwise at rt. The reaction mixture was stirred at reflux overnight, then dissolved in DCM (15 mL) and washed with water. The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 687 mg (77%) of **7** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 2.5, 0.9 Hz, 1H), 7.28 – 7.23 (m, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.30 (s, 3H).

Methyl 5-(bromomethyl)-2-methoxybenzoate (8). To a solution of **7** (728 mg, 3.82 mmol) and perbenzoic acid (20 mg, 0.076 mmol) in CCl_4 (10 mL) was added

N-bromosuccinimide (680 mg, 3.82 mmol). The reaction mixture was stirred at reflux until the starting compound was totally consumed. The solvent was evaporated to give a residue, which was dissolved in DCM (50 mL) and washed with water. The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 840 mg (85%) of **8** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 2.4 Hz, 1H), 7.51 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 4.48 (s, 2H), 3.91 (s, 3H), 3.89 (s, 3H); ESI-MS *m*/*z*: 259.1 $(M+H)^+$.

Methyl 5-(aminomethyl)-2-methoxybenzoate (9). A solution of **8** (130 mg, 0.5 mmol) in NH_3 (12.5 mL, 7 N in MeOH) was stirred at 70°C for 2 hrs. Then the solvent was evaporated to give a residue which was purified by SGC, affording 90 mg (98%) of **9** as an off yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 2.4 Hz, 1H), 7.41 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 5.27 (s, 2H), 3.91 – 3.84 (m, 6H), 3.81 (s, 2H); ESI-MS *m*/*z*: 196.0 $(M+H)^+$.

(*Z*)-Methyl5-(((2-(2-chloro-5-iodobenzoyl)-3-ethoxy-3-oxoprop-1-en-1-yl)amino) methyl)-2-methoxybenzoate (10). A stirred solution of **4b** (283mg, 1 mmol) in EtOH (3 mL) and Et_2O (1 mL) was added dropwise a solution of **9** (0.18, 1.63mmol) in EtOH (0.5 mL). The reaction mixture was stirred at rt for 15 min, then dissolved in DCM (15 mL) and washed with water. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 373 mg (67%) of **10** as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.19 (t, *J* = 8.1 Hz, 1H), 8.32- 8.18 (m, 1H), 7.78 – 7.72 (m, 1H), 7.60 – 7.52 (m, 1H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.44 – 7.38 (m, 1H), 7.09 – 7.00 (m, 2H), 4.60 – 4.54 (m, 2H), 3.98 (q, *J* = 7.1 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 0.94 (t, *J* = 7.1 Hz, 3H).

Ethyl 6-iodo-1-(4-methoxy-3-(methoxycarbonyl)benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (11). To a solution of **10** (373 mg, 0.67 mmol) in dry DMF (2 mL) was added K_2CO_3 (248 mg, 1.8 mmol). The mixture was heated at 100°C for 40 min. After cooling, the reaction mixture was poured into ice-water. The precipitated solid was filtered off, washed with water, and dried to give 331 mg (95%) of **11** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (d, *J* = 2.2 Hz, 1H), 8.57 (s, 1H), 7.80 (dd, *J* = 8.9,

2.2 Hz, 1H), 7.67 (d, *J* = 2.5 Hz, 1H), 7.17 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 5.31 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.91 – 3.87 (m, 6H), 1.42 (t, *J* = 7.2 Hz, 3H).

6-Iodo-1-(4-methoxy-3-(methoxycarbonyl)benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3a). According to the same procedure described for **1a**, **11** (331 mg, 0.64 mmol) was treated with HCl (6N, 5.0 mL) to afford 282 mg (90%) of **3a** as a white solid. Mp: 244-246°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.57 (s, 1H), 8.89 (s, 2H), 7.97 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.69 (d, *J* = 2.4 Hz, 1H), 7.25 – 7.17 (m, 2H), 6.97 (d, *J* = 8.7 Hz, 1H), 5.43 (s, 2H), 3.893 (s, 3H), 3.892 (s, 3H); ESI-MS *m*/*z*: 494.0 $(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{20}H_{16}NO_6NaI$ 515.9920, found 515.9924.

1-(4-Hydroxy-3-(methoxycarbonyl)benzyl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3b). To a stirred solution of **3a** (34 mg, 0.07 mmol) in DCM (5 mL) was added BBr_3 (0.07 mL, 0.28 mmol) in an ice bath. The mixture was stirred at rt for 6 hrs. Saturated $NaHCO_3$ solution (1 mL) was added to quench the reaction. The mixture was then distributed between DCM (20 mL) and H_2O (5 mL). The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by Prep-HPLC (CH_3CN / H_2O (0.5% TFA) = 50 / 50) to give 32 mg (95%) of **3b** as a white solid. Mp: 240-242°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.86 (s, 1H), 8.91 – 8.85 (m, 2H), 7.98 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.65 – 7.62 (m, 1H), 7.32 – 7.27 (m, 2H), 7.02 (d, *J* = 8.7 Hz, 1H), 5.40 (s, 2H), 3.94 (s, 3H); ESI-MS *m*/*z*: 480.2 $(M+H)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{19}H_{14}NO_6I$ 479.9944, found 479.9952.

Methyl-5-((3-(benzylcarbamoyl)-6-iodo-4-oxoquinolin-1(4H)-yl)methyl)-2-methoxybenzoate (3c). According to the same procedure described for **2c**, **3a** (123 mg, 0.25 mmol) was treated with benzylamine (33 mg, 0.3 mmol) to afford 52 mg (35%) of **3c** as a white solid. Mp: 215-216°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.28 (t, *J* = 6.1 Hz, 1H), 8.94 (s, 1H), 8.84 (d, *J* = 2.1 Hz, 1H), 7.85 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.68 (d, *J* = 2.4 Hz, 1H), 7.43 – 7.27 (m, 5H), 7.19 – 7.10 (m, 2H), 6.93 (d, *J* = 8.7 Hz, 1H), 5.38 (s, 2H), 4.70 (d, *J* = 5.8 Hz, 2H), 3.89 (s, 3H), 3.88 (s, 3H); ESI-MS *m*/*z*: 605.1 $(M+Na)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{27}H_{23}N_2O_5NaI$ 605.0549, found

605.0544.

3-(1,1-Diethoxyethyl)benzonitrile (12). A solution of 3-acetylbenzonitrile (725 mg, 5 mmol), p-toluenesulfonic acid (95 mg, 0.5 mmol) and triethoxy methane (1.67 mL, 10 mmol) in EtOH (15 mL) was stirred at rt for 4 hrs. Sodium ethoxide was added and the mixture was filterd through celite pad and the organic layer was concentrated to give 986 mg (90%) of **12** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (q, *J* = 1.7 Hz, 1H), 7.77– 7.71 (m, 1H), 7.61– 7.54 (m, 1H), 7.49– 7.41 (m, 1H), 3.54– 3.41 (m, 2H), 3.37– 3.25 (m, 2H), 1.53 (s, 3H), 1.21 (td, *J* = 7.1, 1.5 Hz, 6H).

(3-(1,1-Diethoxyethyl)phenyl)methanamine (13). To a stirred a solution of **12** (986 mg, 4.45 mmol) in anhydrous THF (10 mL) in an ice bath was added lithium aluminum hydride (513 mg, 13.5 mmol). The mixture was stirred at rt for 1 hr. Saturated NH_4Cl solution (5 mL) was added to quench the reaction and the mixture was dissolved in DCM (30 mL). The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 766 mg (78%) of **13** as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49– 7.45 (m, 1H), 7.43– 7.37 (m, 1H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.26– 7.20 (m, 1H), 3.89 (s, 2H), 3.55– 3.43 (m, 2H), 3.43– 3.31 (m, 2H), 1.81 (bs, 2H), 1.58– 1.52 (m, 3H), 1.26– 1.17 (m, 6H).

Ethyl-1-(3-(1,1-diethoxyethyl)benzyl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (14). According to the same procedure described for **11**, **4b** (150 mg, 0.33 mmol) was treated with **13** (111 mg, 0.5 mmol) and the crude product was used directly in the cyclization step to give 166 mg (90%, two steps) of **14** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (d, *J* = 2.1 Hz, 1H), 8.59 (s, 1H), 7.78 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.38 (s, 1H), 7.36– 7.30 (m, 1H), 7.06 (d, *J* = 8.9 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 5.37 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.51– 3.39 (m, 2H), 3.33– 3.20 (m, 2H), 1.50 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 6H).

Ethyl-1-(3-acetylbenzyl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (15). A solution of **14** (166 mg, 0.302 mmol) in formic acid (6 mL) was stirred at rt for 4 hr. The solvent was evaporated to give **15** as a white solid (148 mg, 100%). ^{1}H NMR

(300 MHz, $CDCl_3$) δ 8.84 (d, *J* = 2.2 Hz, 1H), 8.62 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.85 (s, 1H), 7.80 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.24 (t, *J* = 1.2 Hz, 1H), 7.00 (d, *J* = 8.9 Hz, 1H), 5.42 (s, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 2.59 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H).

(*Z*)-1-(3-(4-Ethoxy-3-hydroxy-4-oxobut-2-enoyl)benzyl)-6-iodo-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (3d). According to the same procedure described for **1a**, **15** (148 mg, 0.31 mmol) was treated with HCl (6N, 1.5 mL) to afford 138 mg (98%) of a white solid which was used directly for the next step. A solution of the solid obtained above (138 mg, 0.31 mmol), NaOMe (50 mg, 0.93 mmol) and oxalyl acetate (84 uL, 0.62 mmol) in THF (1.5 mL) was stirred in a microwave tube at 50℃ for 30 min. The reaction mixture was quenched with saturated NH_4Cl solution (5 mL) and dissolved in DCM (20 mL). The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by Prep-HPLC (CH_3CN / H_2O (0.5% TFA) = 40 / 60) to give 24 mg (35%) of **3d** as a light yellow solid. Mp: 166-168°C; ^{1}H NMR (300 MHz, CD_3OD) δ 9.17 (s, 1H), 8.78 (d, *J* = 1.9 Hz, 1H), 8.08 – 7.94 (m, 3H), 7.59 – 7.43 (m, 3H), 7.06 (s, 1H), 5.82 (s, 2H), 4.35 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 190.2, 177.4, 165.3, 161.6, 150.6, 142.3, 140.6, 139.5, 136.3, 135.7, 135.2, 131.9, 130.0, 128.1, 127.8, 126.3, 120.5, 109.5, 97.9, 90.3, 62.2, 57.0, 13.6; ESI-MS *m/z*: 548.1$(M+H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{23}H_{18}NO_7NaI$ 570.0026, found 570.0033.

(*Z*)-1-(3-(3-Carboxy-3-hydroxyacryloyl)benzyl)-6-iodo-4-oxo-1,4-dihydroquinolin e-3-carboxylic acid (3e). To a solution of **3d** (12 mg, 0.021 mmol) in THF / EtOH (1 / 1, v / v, 1 mL) was added NaOH (42 uL, 0.042 mmol) at rt. The solution was stirred for 30 min. The solvent was evaporated and the reidue was dissolved in water (5 mL). The solution was extracted with DCM (3 x 5 mL). The aqueous phase was collected and adjusted to pH 2~3 with HCl (1N). Then the mixture was extracted with DCM (3x10 mL). The organic phases were combined, washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give a residue which was purified by Prep-HPLC (CH_3CN / H_2O (0.5% TFA)= 30 / 70) to give 3.8 mg (34%) of **3e** as a yellow solid. Mp: 163-165°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.87 (d, *J* =

1.4 Hz, 1H), 7.97 (dd, *J* = 15.7, 6.1 Hz, 3H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.10 (s, 1H), 5.67 (s, 2H); ESI-MS *m/z*: 518.0$(M-H)^+$; HRMS (ESI) $(M+Na)^+$: calcd for $C_{21}H_{14}NO_7NaI$ 541.9713, found 541.9704.

Methyl 2-(3-((tert-butoxycarbonyl)amino)propoxy)-6-hydroxybenzoate (16). A solution of methyl 2,6-dihydroxybenzoate (274 mg, 1.63 mmol), *tert*-butyl (3-bromopropyl)carbamate (387 mg, 1.63 mmol) and K_2CO_3 (338 mg, 2.44 mmol) in DMF (10 mL) was stirred at 80°C until the reaction completed. The solvent was evaporated and the residue was partioned between DCM (10 mL) and H_2O (5 mL). The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography to afford target compound **16** as a white solid (342 mg，65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.55 (s, 1H), 7.32 (t, *J* = 8.4 Hz, 1H), 6.61 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.39 (d, *J* = 8.6 Hz, 1H), 4.07 (t, *J* = 5.5 Hz, 2H), 3.99 (s, 3H), 3.38 (q, *J* = 5.7 Hz, 2H), 2.03 (p, *J* = 6.0 Hz, 2H), 1.43 (s, 9H).

Methyl 2-(3-aminopropoxy)-6-hydroxybenzoate (17). To a solution of **16** (342 mg, 1.05 mmol) in DCM (5 mL) was added TFA (0.5 mL) at rt. The mixture was stirred for 3 h. The solvent was then evaporated to give **17** as a light brown solid (236 mg, 100%) which was used directly for the next step.

Methyl 2-(3-(1-benzyl-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamido)propoxy)-6-hydroxybenzoate (3f). According to the same procedure described for **2c**, **1g** (30 mg, 0.1 mmol) was treated with **17** (26 mg, 0.12 mmol) to afford 42 mg (83%) of **3f** as a white solid. Mp: 155-157°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.52 (s, 1H), 10.10 – 10.02 (m, 1H), 8.90 (s, 1H), 8.52 (dd, *J* = 9.0, 6.4 Hz, 1H), 7.43 – 7.28 (m, 4H), 7.21 – 7.12 (m, 3H), 7.06 (dd, *J* = 10.3, 2.0 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.42 (d, *J* = 8.1 Hz, 1H), 5.40 (s, 2H), 4.13 (t, *J* = 6.0 Hz, 2H), 3.99 (s, 3H), 3.73 (dd, *J* = 13.0, 6.6 Hz, 2H), 2.25 – 2.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 171.8, 165.3 (d, *J* = 252 Hz, 1H), 164.8, 163.5, 160.2, 148.9, 140.9 (d, *J* = 11.4 Hz, 1H), 135.1, 133.6, 130.3 (d, *J* = 10.6 Hz, 1H), 129.5, 128.9, 126.1, 124.7, 114.2, 114.0, 112.6, 110.0,

103.3 (d, *J* = 5.6 Hz, 1H), 103.2, 66.4, 57.9, 52.4, 36.3, 29.5; ESI-MS *m/z*: 505.3 $(M+H)^+$; HRMS (ESI) $[M+H]^+$: calcd for $C_{28}H_{25}N_2O_6F$ 505.1775, found 505.1796.

Methyl 2-(3-(1-(4-fluorobenzyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxamido)propoxy)-6-hydroxybenzoate (3g). According to the same procedure described for **2c**, **1e** (34 mg, 0.1 mmol) was treated with **17** (26 mg, 0.12 mmol) to afford 36 mg (67%) of **3g** as a white solid. Mp: 218-221°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 9.72 (t, *J* = 5.9 Hz, 1H), 9.17 (s, 1H), 9.02 (d, *J* = 2.8 Hz, 1H), 8.49 (dd, *J* = 9.4, 2.8 Hz, 1H), 7.97 (d, *J* = 9.5 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.22 – 7.15 (m, 3H), 6.51 (dd, *J* = 13.3, 8.2 Hz, 2H), 5.83 (s, 2H), 4.04 (t, *J* = 6.1 Hz, 2H), 3.78 (s, 3H), 3.48 (dd, *J* = 12.3, 7.0 Hz, 2H), 1.95 (dt, *J* = 13.0, 6.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.7, 167.3, 163.8, 162.2 (d, *J* = 243.1 Hz, 1H), 157.1, 156.2 (d, *J* = 16.3 Hz, 1H), 150.7, 144.3, 143.1, 131.9, 131.7, 129.3 (d, *J* = 7.6 Hz, 1H), 127.6, 127.2, 122.7, 120.3, 116.3 (d, *J* = 20.8 Hz, 1H), 113.3, 111.6, 109.0, 103.6, 66.3, 56.0, 52.2, 36.0, 29.5; EI-MS *m/z*: 549 (M^+); HRMS (EI) $(M)^+$: calcd for $C_{28}H_{24}N_3FO_8$ 549.1649, found 549.1654.

Methyl 2-(benzyloxy)-6-hydroxybenzoate (18). A solution of methyl 2,6-dihydroxybenzoate (840 mg, 5 mol), K_2CO_3 (1.38 g, 10 mmol) and benzyl bromide (0.66 ml, 5.5 mmol) in anhydrous CH_3CN (15 mL) was stirred at 60°C overnight. The solvent was evaporated to give a residue, which was dissolved in DCM (50 mL) and washed with water. The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 500 mg (40%) of **18** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 7.50 – 7.46 (m, 2H), 7.43 (dd, *J* = 8.0, 6.5 Hz, 2H), 7.36 (t, *J* = 8.3 Hz, 1H), 6.65 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.51 (dd, *J* = 8.3, 1.0 Hz, 2H), 5.14 (s, 2H), 3.97 (s, 3H).

Methyl 2-(benzyloxy)-6-(3-hydroxypropoxy)benzoate (19). To a stirred solution of **18** (300 mg, 1.16 mmol) and K_2CO_3 (320 mg, 2.32 mmol) in anhydrous CH_3CN (3 mL) was added 3-bromopropan-1-ol (0.13 mL, 1.39 mmol) and the mixture was stirred at 80°C overnight. The solvent was evaporated to give a residue, which was dissolved in DCM (150 mL) and washed with water. The organic phase was combined,

washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 300 mg (95%) of **19** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 –7.33 (m, 4H), 7.33 (dd, *J* = 6.1, 2.6 Hz, 1H), 7.25 –7.21 (m, 1H), 6.61 (dd, *J* = 8.4, 5.5 Hz, 2H), 5.13 (s, 2H), 4.19 (t, *J* = 5.7 Hz, 2H), 3.91 (s, 3H), 3.86 (t, *J* = 5.6 Hz, 2H), 2.05 (dt, *J* = 11.3, 5.3 Hz, 2H).

Methyl 2-(benzyloxy)-6-(3-oxopropoxy)benzoate (20). A solution of **19** (158 mg, 0.5 mmol) and Dess-Martin oxidant (318 mg, 0.75 mmol) in anhydrous DCM (2 mL) was stirred at rt for 3 hrs. The solvent was evaporated to give a residue, which was dissolved in DCM (150 mL) and washed with water. The organic phase was combined, washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by SGC to give 82 mg (40%) of **20** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.83 (t, *J* = 1.6 Hz, 1H), 7.40 –7.30 (m, 5H), 7.27 (t, *J* = 8.4 Hz, 1H), 6.62 (dd, *J* = 8.4, 4.4 Hz, 2H), 5.14 (s, 2H), 4.37 (t, *J* = 6.2 Hz, 2H), 3.88 (s, 3H), 2.90 (td, *J* = 6.2, 1.6 Hz, 2H).

6-(4-(3-(3-(Benzyloxy)-2-(methoxycarbonyl)phenoxy)propyl)piperazin-1-yl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3h). According to the same procedure described for **1k**, **1a** (23 mg, 0.09 mmol) was treated with piperazine followed by a solution of **20** (34 mg, 0.11 mmol) in DCE (0.5 mL) to afford 20 mg (37%) of **3h** as a light yellow solid. Mp: 173-174°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.00 (d, *J* = 9.4 Hz, 1H), 7.82 (d, *J* = 2.7 Hz, 1H), 7.50 (dd, *J* = 9.3, 2.8 Hz, 1H), 7.41 – 7.36 (m, 4H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.25 (t, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 2H), 5.14 (s, 2H), 4.11 (t, *J* = 6.0 Hz, 2H), 3.93 (s, 3H), 3.65 – 3.57 (m, 1H), 3.44 – 3.36 (m, 4H), 2.75 – 2.67 (m, 4H), 2.63 (dd, *J* = 11.9, 6.5 Hz, 2H), 2.04 (dd, *J* = 14.3, 8.0 Hz, 2H), 1.39 (dd, *J* = 13.5, 6.7 Hz, 2H), 1.24 – 1.18 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.2, 167.6, 167.0, 156.7, 156.4, 149.5, 146.0, 136.8, 134.2, 131.0, 128.5, 127.8, 126.8, 123.1, 118.2, 114.0, 109.1, 107.7, 105.6, 105.3, 70.4, 66.8, 54.8, 52.9, 52.3, 48.4, 35.3, 31.9, 29.7, 26.6, 22.7, 8.1; ESI-MS *m/z*: 612.4 $(M+H)^+$; HRMS (ESI) $(M+H)^+$: calcd for $C_{35}H_{37}N_3O_7$ 612.2710, found 612.2715.

PTP1B and related PTPs biological assay

A colorimetric assay to measure inhibition against PTP1B and TCPTP was performed in 96-well plates. Briefly, the tested compounds were solubilized in DMSO and serially diluted into concentrations for the inhibitory test. The assays were carried out in a final volume of 100 μL containing 50 mmol/L MOPS, pH 6.5, 2 mmol/L pNPP, 30 nmol/L GST-PTP1B or GST-TCPTP, and 2% DMSO, and the catalysis of pNPP was continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 3 min at 30 °C. The IC_{50} value was calculated from the nonlinear curve fitting of the percent inhibition [inhibition (%)] *vs* the inhibitor concentration using the following equation: %inhibition=100/(1+(IC_{50}/[I]k),where k is the Hill coefficient. To study the inhibition on the other PTPs family members, SHP1, SHP2 and LAR were prepared and assays were performed according to procedures described previously.[28, 32] Briefly, the enzymatic activity of the SHP1, SHP2 and LAR were determined at 30 °C by monitoring the dephosphorylation of substrate 3-o-methylfluorescein phosphate (OMFP), product was then detected at a 485 nm excitation wavelength and 530 nm emission wavelength by the EnVision multilabel plate reader (Perkin-Elmer Life Sciences, Boston, MA, USA). The assays were carried out in a final volume of 50 μL containing 50 mmol/L MOPS, pH 6.5, 10 μmol/L OMFP, 20 nmol/L recombinant enzyme, 2 mmol/L dithiothreitol , 1 mmol/L EDTA, and 2% DMSO. The initial rate of the dephosphorylation was presented by the early linear region of the enzymatic reaction kinetic curve, the inhibitory activity of the compound was continuously monitored,

Characterization of the inhibitor on enzyme kinetics

To characterize the inhibitor of PTP1B, the assay was carried out in a 100 μL system containing 50 mmol/L MOPS, pH 6.5, 30 nmol/L PTP1B, pNPP in 2-fold dilution from 80 mmol/L, and different concentrations of the inhibitor. In the presence of the competitive inhibitor, the Michaelis-Menten equation is described as 1/v = (Km/[Vmax[S]])(1+[I]/Ki)+1/Vmax, where Km is the Michaelis constant, v is the initial rate, Vmax is the maximum rate, and [S] is the substrate concentration. The Ki value was obtained by the linear replot of apparent Km/Vmax (slope) from the primary reciprocal plot versus the inhibitor concentration [I] according to the equation

Km/Vmax = 1+ [I]/Ki.

Effect of PTP1B inhibitors on the phosphorylation level of CHO/*h*IR cell line.

CHO/*h*IR cells were cultured in F12 medium including 10% (V/V) FBS, 100 units/mL Penicillin and 100 μg/mL streptomycin with 5% CO_2 at 37°C. Cells serum free starved for 2 hours were incubated with compounds for 1 hour, then insulin (10 nM, Lilly) was added stimulation for 10 min before harvested. After washing twice with precooled 1X PBS, cells were lysed with 1 x SDS loading buffer. Cell lysates were subjected to 8% SDS-polyarylamide gel and transferred to nitrocellulose membranes, the membranes were blocked for 2 hours with 5% BSA (W/V). The primary antibodies incubated overnight at 4°C and secondary antibodies for 1 hour at room temperature. The primary antibody p-Tyr (PY20) was from Santa Cruz and β-actin from Sigma, secondary antibody was from Jackson Immuno Research.

Molecular modeling

The crystal structures of PTP1B with the active site of the enzyme in WPD loop open conformation (PDB: 3QKP),[30] and in WPD loop close conformation (PDB: 1PXH),[31] was recovered from Brookhaven Protein Database. The missing atoms and residues were modeled in Sybyl 6.8.17. The kinds of atomic charges were taken as Kollman-united-atom[33] for the macromolecule and Gasteiger–Marsili[34] for the inhibitor.

To establish the binding mode of compound **3d** to the active site of PTP1B, the advanced docking program Autodock 3.0.3[35] was used to automatically dock the inhibitor to the enzyme. The Lamarckian genetic algorithm (LGA) was applied to deal with the inhibitor–enzyme interaction. A Solis and Wets local search performed the energy minimization on a user-specified proportion of the population. The docked conformations of the inhibitor were generated after a reasonable number of evaluations. The lowest energy conformation was selected as the ligand-protein binding mode, then the binding free energy of ligand-protein complexes was calculated by applying the MM-PBSA package in Discovery

Studio 3.5 software.

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Graphical Abstracts (Table of Contents)

4-Quinolone-3-carboxylic acids as cell-permeable inhibitors of protein tyrosine phosphatase 1B

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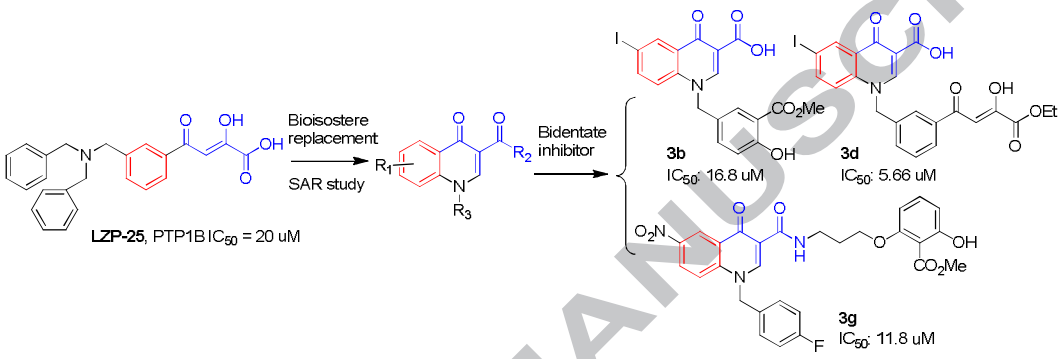

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