Published in final edited form as:
Bioorg Med Chem Lett. 2011 July 1; 21(13): 4036–4040. doi:10.1016/j.bmcl.2011.04.129.

Discovery and optimization of potent and selective benzonaphthyridinone analogs as small molecule mTOR inhibitors with improved mouse microsome stability

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Abstract

Starting from small molecule mTOR inhibitor Torin1, replacement of the piperazine ring with a phenyl ring resulted in a new series of mTOR inhibitors (as exemplified by **10**) that showed superior potency and selectivity for mTOR, along with significantly improved mouse liver microsome stability and a longer *in vivo* half-life.

Keywords

mTOR; PI3K; Torin1

Mammalian target of rapamycin (mTOR) is a key node of the PI3K/Akt/mTOR signal transduction pathway. mTOR pathway has been found to be deregulated in a variety of cancers and has been extensively studied as an oncology drug discovery target.[1,2,3,4] Upon activation by external or internal stimuli such as growth factors, nutrients, stress, and energy, mTOR regulates cell growth, proliferation, metabolism, and autophagy through the mTORC1 and mTORC2 complexes by phosphorylation of downstream targets S6K, 4EBP1, and Akt.[5] The successful clinical application of rapamycin in renal cell carcinoma has validated mTOR as an anti-cancer drug discovery target. However, a lack of activity against mTORC2, incomplete inhibition of mTORC1 function and negative reactivation of Akt through the S6K/IRS1 pathway may explain the limited clinical efficacy observed with rapamycin related compounds (rapalogs) [6,7,8] Currently, there is significant interest in the development and characterization of ATP-competitive mTOR inhibitors which would result in complete inhibition of both mTORC1 and mTORC2.

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mTOR is a member of the PI3K kinase family, which consists of the PI3Ks, DNA-PK, ATR, ATM, and SMG-1.[9] PI3Ks, especially PI3Kα, have been found to be hyperactivated in a wide spectrum of cancers, and are currently being evaluated as an anti-cancer drug target in the clinic.[1] The structural similarity between PI3Ks and mTOR endowed many early PI3K or mTOR inhibitors, like PI-103 and BEZ-235, with dual activity against both targets.[10,11] Because PI3Ks are upstream regulators of Akt, a central hub of many critical cellular processes, it is believed that mTOR-selective inhibitors may demonstrate improved toxicology relative to dual PI3K/mTOR inhibitors. Many of the recently disclosed mTOR inhibitors, including Torin1, have demonstrated that it is possible to selectively inhibit mTOR versus PI3Ks.[12–22]

We recently prepared a series of tricyclic benzonaphthyridinones, exemplified by Torin1, as highly potent and selective mTOR inhibitors. Torin1 has more than 800-fold selectivity for mTOR relative to PI3Ks in cellular assays and has no other significant protein kinase off-targets among the 450 kinases profiled by KINOMEscan™.[12] Torin1 exhibited anti-tumor activity in a U87-MG mouse xenograft that correlated with target inhibition. However, Torin1 exhibits poor mouse microsome stability and a short *in vivo* half-life which limit its utility as a pharmacological agent *in vivo*. In this letter, we describe our efforts to improve the pharmacokinetic properties of Torin1 by replacing the metabolically labile phenylpiperazine moiety with a biphenyl system to yield compounds such as **10** (Figure 1).

The preparation of compound **10** and analogs is illustrated in Scheme 1. Ethyl-4,6-dichloroquinoline-3-carboxylate (**1**) was subjected to nucleophilic substitution with 4-bromo-3-trifluoromethaneaniline (**2**) to afford compound **3**. Reduction of ethyl ester **3** with $NaBH_4$ generated benzyl alcohol **4**, which was subjected to benzylic oxidation with MnO_2 and olefination/cyclization *in situ* to furnish general intermediate **5**. Pinacol boronic ester **6** was obtained from 4-bromo-benzonic acid **(8)** via Suzuki coupling and amide formation. A bromine-selective Suzuki coupling was used to install the benzoate side chain (or other substituent in the analogs) to afford the intermediate **7**. Finally, the quinoline side chain (or other substituent in the analogs) was attached at the chlorine position using more vigorous Suzuki coupling conditions to afford the final product illustrated by compound **10**.

Following this general synthetic strategy, a focused library of compounds was generated by varying the benzoate side chain, quinoline side chain and CF_3/CH_3 moiety. The selected compounds were evaluated in parallel in biochemical assays with mTORC1 complex in cellular assays using a mouse embryonic fibroblast (MEF) cell line by examining the phosphorylation status of mTOR downstream targets such as S6K (T389), and for PI3K activity with the Akt S473D PC-3 cell line by examining the phosphorylation status of AktT308. The results are summarized in Table 1.

Based on the Torin1 series, we first retained the quinoline side at the $\mathbf{R^1}$ position, which was presumably responsible for the selectivity and potency against mTOR. Replacing the phenylpiperazine ring with a biphenyl system provided compounds that demonstrated excellent potency against mTOR and selectivity relative to PI3K. Methyl amide **11**, THP-protected benzyl alcohol **12**, benzyl alcohol **13,** carboxylic acid **14**, and primary amide **16** all demonstrated the same level of potency against mTOR (EC_{50} values between 15–30 nM) and the same selectivity over PI3K (EC_{50} over 300 nM). The methyl ester **15** lost a significant amount of activity against mTOR, suggesting that the active members of this series of compounds possess hydrogen bond donors through either an –OH, –COOH. or –$CONH_2$ functionality. *N*-methyl-piperidin-4-amine amide **10** exhibited the best potency against mTOR (EC_{50} = 5 nM, the same as Torin1) and more than 200-fold selectivity over PI3K. The compounds derived from replacing the quinoline moiety with aminopyrimidine (**17**) or pyrazole (**18**) systems were slightly less potent against mTOR, indicating that the

inner hydrophobic pocket required a larger substituent. In order to reduce the molecular weight and obtain better solubility, while the $\mathbf{R^2}$ fragment was kept as methyl-piperidin-4-amine, the CF_3 group was replaced with a CH_3 group and the $\mathbf{R^1}$ side chain was varied to include a pyrazole (**20**), methylpyrazole (**21**), 7-azaindole (**22**), hydrogen (**23**) and aminopyridine (**24**). However, all of these analogs failed to produce the same level of potency against mTOR, although the selectivity over PI3Ks was retained. Compound **19**, which bears quinoline side chain at the $\mathbf{R^1}$ position, retained the same potency and selectivity against mTOR as Torin1 and compound **10**. This reaffirmed that quinoline was the best side chain to occupy the inner hydrophobic pocket, and that the hydrogen bond provided by the –OH or $–NH_2$ was critical to maintain potency.

With the best activity and selectivity, compounds **10** and **19** were chosen for mouse stability study, single point CYP450 inhibition test (Table 2), and *in vivo* mouse pharmacokinetic analysis (Table 3). In comparison to Torin1, compounds **10** and **19** demonstrated significant improvements in stability in the mouse microsome assay (46 and 42 min, respectively), where both were subjected to NADPH-dependent metabolism. In the single point CYP450 metabolic enzyme inhibitory assay, compound **10** showed more than 60% and 50% inhibition at 10 μM against the major metabolic enzymes CYP3A4 and CYP2D6, respectively, while compound **19** weaker inhibition (34% and 24%, respectively). Further investigation of the ability of these compounds to inhibit metabolism are warranted prior to performing combination studies.

The improved microsome stability profile of compounds **10** and **19** encouraged us to evaluate their *in vivo* pharmacokinetic properties. Upon intravenous (7.5% NMP and 40% PEG400 in water) and oral (0.1% v/v Tween-80, 0.5% w/v NaCMC in water) administration, compound **10** demonstrated superior pharmacokinetic properties relative to compound **19**, although both were significantly better than Torin1.[12] The half-life was improved to 3.6 h (**10**) and 1.8 h (**19**) from that of Torin1 (0.5 h). The bioavailability of compound **10** (10.1%) was double that of Torin1 (5.5%), while the bioavailability of compound **19** was 5.4%. Compound **10** also demonstrated much better exposure using both IV and PO delivery routes compared to Torin1(1388/1411 versus 720/396 hr*ng/mL). Other pharmacokinetic properties such as clearance rate (11.9 versus 23.0 mL/min/Kg) and volume of distribution (1.95 versus 0.59 L/Kg) were also superior to those of Torin1. The slower T_{max} of compound **10** (4 h) compared to compound **19** (1 h) and Torin1 (0.5 h) was indicative of poor solubility and/or slow absorption.

To evaluate the kinase selectivity of compound **10**, it was subjected to the Ambit kinome-wide screen using KINOMEscan™ technology. The assay showed that compound **10** was very selective and did not strongly hit any other protein kinases among the 353 kianses tested, except for several PI3K family lipid kinases (Figure 2, Table 4).

Compound **10** was evaluated in an *in vivo* pharmacodynamic study, where it exhibited significant inhibitory activity against the downstream targets of mTOR, S6K, and Akt, and blocked 80–90% phosphorylation of S6K (T389) and pAkt (S473) in liver and lung tissues even after 6h at a dosage of 20 mg/kg

In summary, starting from Torin1, replacement of the metabolically labile 4-amino-phenylpiperazine moiety with a biphenyl system provided a new series of inhibitors that were exemplified by compound **10**, which demonstrated significant improvements in mouse microsome stability and *in vivo* pharmacokinetic properties. Compound **10** is a potent and selective mTOR inhibitor suitable for use in cell culture and *in vivo*. Further elaboration of this scaffold class to improve the drug-like properties will be reported in due-course.

Acknowledgments

The authors thank Dr. Michael Cameron (Scripps Florida) for the mouse microsome stability studies. The authors also thank the Life Technologies Corporation (Invitrogen) SelectScreen® Kinase profiling service for performing enzymatic biochemical kinase profiling and Ambit Biosciences for performing KINOMEscan™ profiling.

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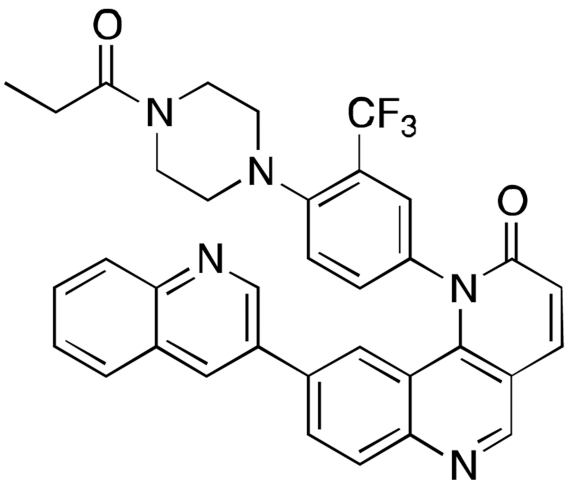
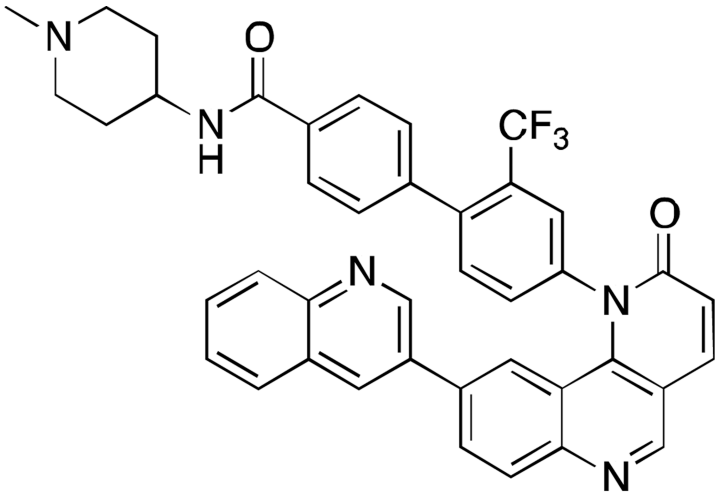
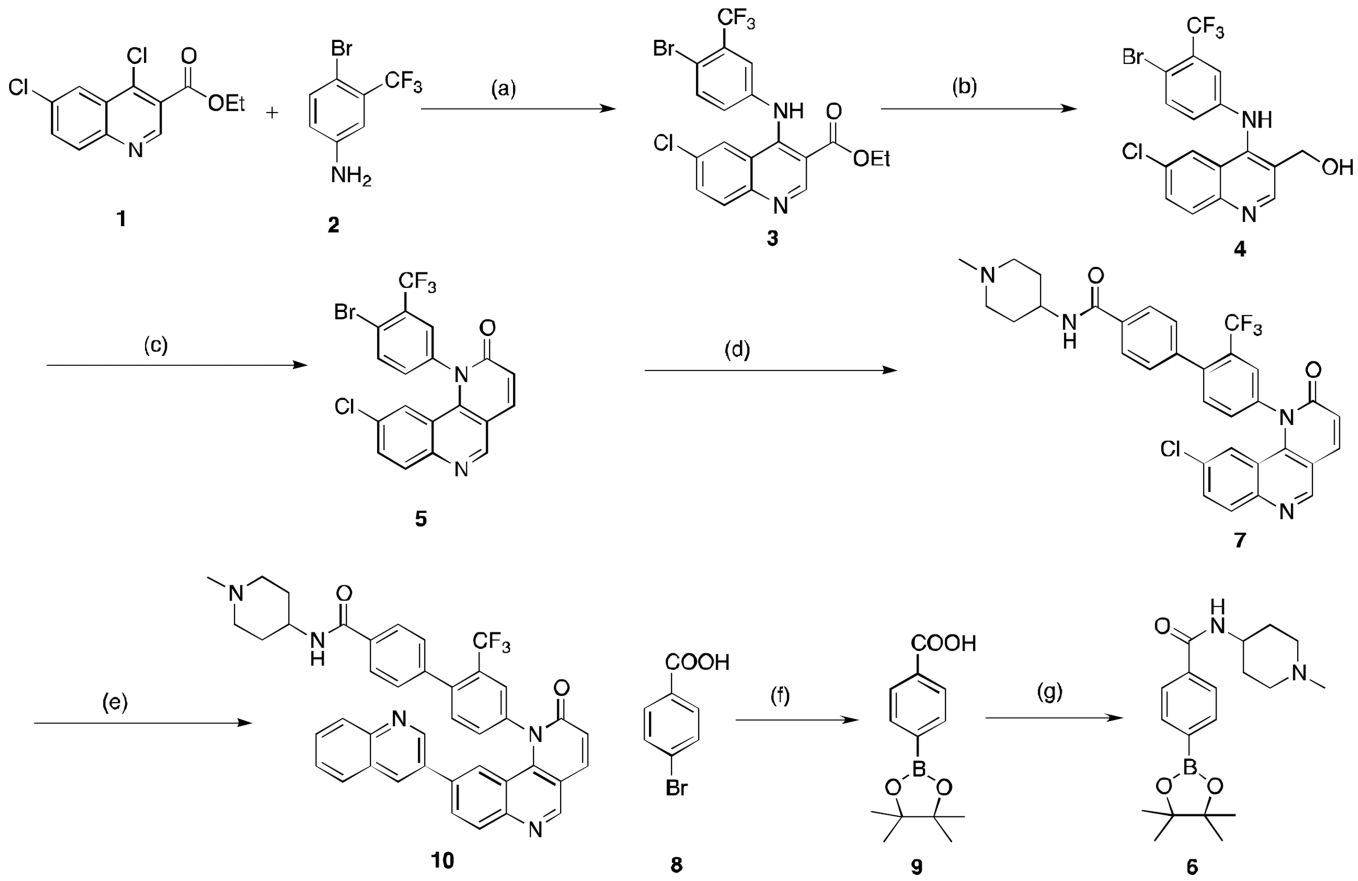


Torin1



10

Figure 1.
Structures of Torin1 and **10**.

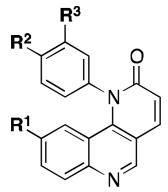


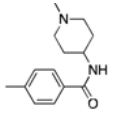
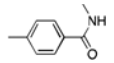
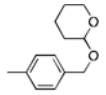
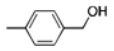
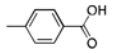
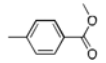
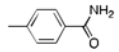
Scheme 1.

Reagents and conditions: (a) 1,4-dioxane, 85 °C, 4 h, 80% yield; (b) $NaBH_4$, EtOH, rt, 4 h, 50% yield; (c) MnO_2, CH_2Cl_2, rt, 2 h; then triethylphosphonoacetate, K_2CO_3, EtOH, 100°C, 12 h, 60% yield; (d) **6**, $PdCl_2(Ph_3P)_2$, Na_2CO_3, 1,4-dioxane, 80°C, 2 h, 60% yield; (e) quinoline-3-boronic acid, $PdCl_2(Ph_3P)_2$, t-Bu-Xphos, Na_2CO_3, 1,4-dioxane, 100°C, 4 h, 50% yield; (f) $PdCl_2$(dppf), bis(pinacolato)diboron, KOAc, 1,4-dioxane, 80°C, 12 h; crude material was used without purification; (g) HATU, THF, 1-methyl-piperidin-4-amine, diisopropylethylamine, rt, 50% over 2 steps.

Table 1

Data from biochemical and cellular assays.[a]



Entry	R^1	R^2	R^3	mTORC1 biochemical IC_{50}(nM)	mTOR cellular EC_{50}(nM)	PI3K cellular EC_{50}(nM)
10			CF_3	21.7	5	1000
11			CF_3	10.2	15	>300
12			CF_3	188	25	>300
13			CF_3	17.3	30	>300
14			CF_3	22.9	20	>300
15			CF_3	299	200	>300
16			CF_3	56.3	25	>300

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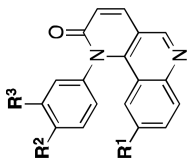
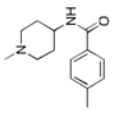
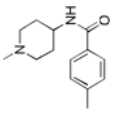
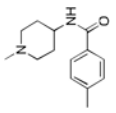
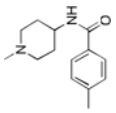
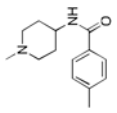
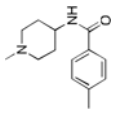


Entry	R^1	R^2	R^3	mTORC1 biochemical IC_{50}(nM)	mTOR cellular EC_{50}(nM)	PI3K cellular EC_{50}(nM)
17			CF_3	43.2	10	1000
18			CF_3	14.1	10	1000
19			CH_3	14.5	1	1000
20			CH_3	38.1	150	>1250
21			CH_3	283	150	>1250
22			CH_3	18.9	25	1000

Entry	R^1	R^2	R^3	mTORC1 biochemical IC_{50}(nM)	mTOR cellular EC_{50}(nM)	PI3K cellular EC_{50}(nM)
23	H		CH_3	>3000	>1000	>1250
24			CH_3	39.8	50	>1250

[a] IC_{50} determinations are the mean of two of independent measurements with a standard error of <20%.

Table 2

Mouse microsome stability and CYP450 inhibition results.

Compound	Mouse microsome stability (min)	NADPH-dependent	CYP3A4 % inhibition (10 μM)	CYP2D6 %inhibition (10 μM)
10	46	Y	61	51
19	42	Y	34	24
Torin1	1.3	N	ND	ND

Table 3

In vivo mouse pharmacokinetic data.

Compound	C_{max} (ng/mL) i.v./P.O.	T_{max} (h) i.v./P.O	AUC(0-∞) (hr*ng/ml) i.v./P.O.	$T_{1/2}$ (h) i.v./P.O.	CL (mL/min/kg) i.v./P.O.	Vss (L/kg) i.v./P.O.	F (%) i.v./P.O.
10	1408/191	-/4.0	1388/1411	3.61/-	11.9/-	1.95/-	-/10.1
19	1046/115	-/1	737/391	1.85/-	22.6/-	1.82/-	-/5.4
Torin1	2757/223	-/0.25	720/396	0.5/0.79	23.0/-	0.59/-	/5.49

Table 4

Ambit and Invitrogen profiles of compound **10** against PIKKs

Kinase symbol	Invitrogen IC_{50} (nM)	Ambit score (%)
PI4Kα	>10000	ND
PI4Kβ	3440	ND
PI3K-C2α	1110	ND
PI3K-C2β	437	0.65
hVPS34	170	ND
P110α/P85α	922	0.1
P110δ/P85α	1170	2
P110γ	1170	0.05
mTOR	3.01	0