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Article

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Celgene Corporation, 10300 Campus Pointe Drive, Suite 100, San Diego, California 92121.

Running Title: Discovery of CC-223

Keywords: Mammalian target of rapamycin, mTOR kinase, kinase inhibitors, PI3K/AKT/mTOR pathway, oncology

Abstract.

We report here the synthesis and structure-activity relationship (SAR) of a novel series of mammalian target of rapamycin (mTOR) kinase inhibitors. A series of 4,6- or 1,7-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-ones were optimized for in vivo efficacy. These efforts resulted in the identification of compounds with excellent mTOR kinase inhibitory potency, with exquisite kinase selectivity over the related lipid kinase PI3K. The improved PK properties of this series allowed for exploration of in vivo efficacy and ultimately the selection of CC-223 for clinical development.

Introduction.

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway is frequently disgregulated in human cancers through multiple mechanisms, including loss of function mutations or promoter hypermethylation of the tumor suppressor PTEN or activating mutations of the PIK3CA oncogene.[1] The mammalian target of rapamycin (mTOR) kinase is found in two distinct multi-protein signaling complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2), which function as critical mediators of the PI3K/AKT pathway.[2] While mTORC1 is responsible for regulating protein synthesis and growth,[3] mTORC2 has been shown to phosphorylate and activate AKT,[4] a key kinase in the control of cell growth, metabolism and survival. Rapamycin analogs such as temsirolimus and everolimus, allosteric inhibitors that generally do not inhibit mTORC2 and have been shown to only partially inhibit the mTORC1 complex have achieved some clinical success, though it is hypothesized that mTOR kinase (TORK) inhibitors, blocking both mTORC1 and mTORC2 signaling, will have expanded therapeutic potential.[5] An attractive drug target, mTOR kinase has been the focus of much investigation in the pharmaceutical industry and ATP-competitive inhibitors selective for mTOR kinase relative to the related PI3K-α, such as OSI-027[6], AZD8055[7], AZD2014[7], MLN0128[8], CC-115[9] and CC-223[10,11], have entered clinical trials.

We have previously reported the identification of a series of 1,6-substituted imidazo[4,5-b]pyrazin-2-one based mTOR kinase inhibitors following an HTS campaign and initial optimizations.[12] Optimization in this series provided analogs with excellent potency and selectivity over PI3Kα, such as **1** (CC214-1) and **2** (Figure 1A). Further optimization to achieve good oral PK properties however, proved to be a challenge. We therefore evaluated core modifications to expand the chemical matter available to the program. Insertion of a methylene

unit in the imidazo ring led to the identification of two new series of potent and selective mTOR kinase inhibitors (Figure 1B). We have previously disclosed the initial results of our first ring expansion effort resulting in the identification of an in vivo tool compound CC214-2 (**26**)[13], here we describe the SAR exploration in both ring expansion series and the selection of CC-223 for clinical development.

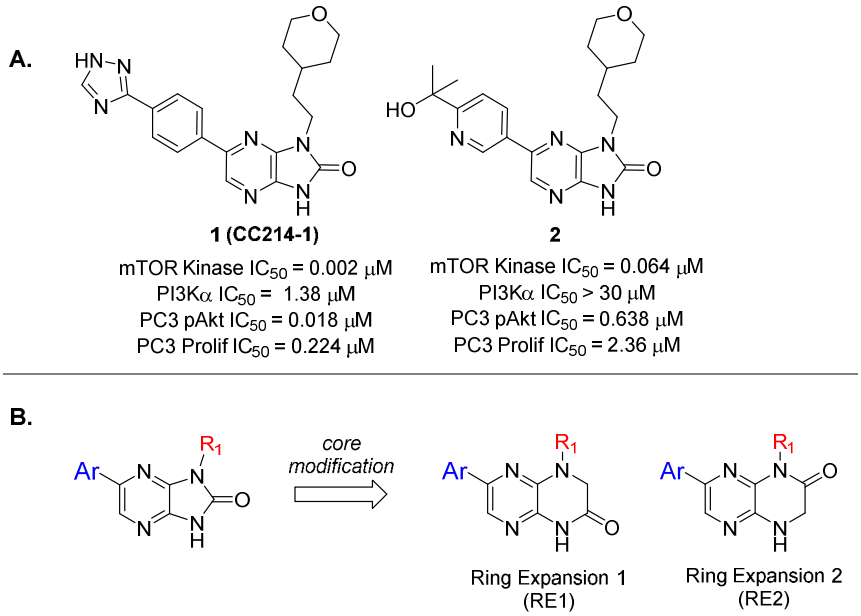


Figure 1. (**A)** Representative analogs from initial compound series. (**B)** Core modification of the imidazo[4,5-b]pyrazin-2-ones to give 3,4-dihydropyrazino[2,3-b]pyrazin-2(*1H*)-ones.

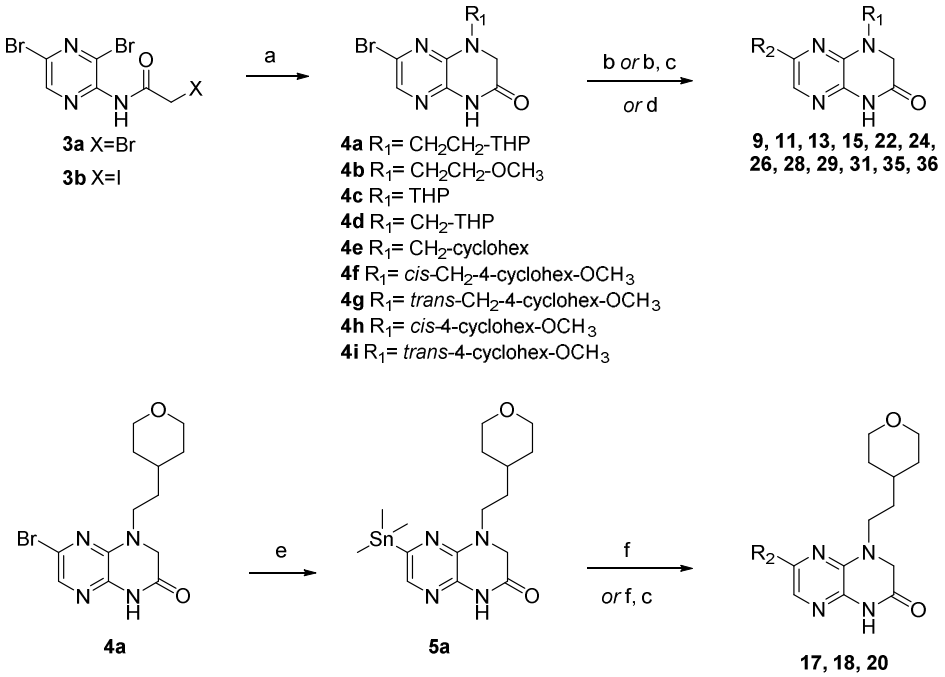
Chemistry

Compounds from the 4,6-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-one (RE1) series were synthesized by the methods in Scheme 1. Amine addition to either 2-bromo- **(3a)** or 2-iodo-*N*-(3,5-dibromopyrazin-2-yl)acetamide **(3b**) afforded the desired addition and ring closure products **4a-i**. Three methods were employed to achieve the aryl substitution at C6 of the 3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-one core. In one method, Suzuki coupling of intermediate **4a** with aryl boronic acid pinacol esters, followed by acid catalyzed deprotection,

when relevant, afforded the desired compounds **9**, **13** and **15**. Alternatively, **4a-i** were subjected to Stille coupling with 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol to afford analogs **11, 22, 24, 26, 28, 29, 31, 35,** and **36**. Finally, bromide **4a** was converted to stannane **5a**. Stille coupling of **5a** with aryl bromides, followed by acid catalyzed deprotection, when relevant, gave compounds **17, 18** and **20**.

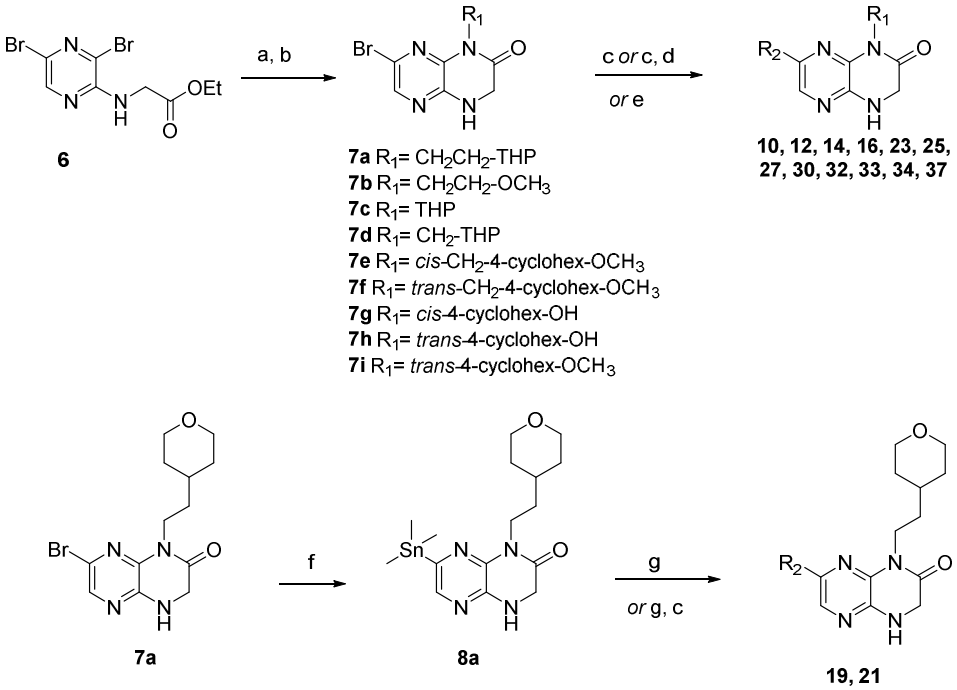
Compounds from the 1,7-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-one (RE2) series were synthesized by the methods in Scheme 2. Addition of amines to ethyl (3,5-dibromopyrazin-2-yl)glycinate **(6)**, followed by acid catalyzed ring closure gave the desired intermediates **(7a-i)**. As with the RE1 analogs, three methods were employed in completing the aryl substitution, now at the C7 of the 3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-one core. Suzuki coupling of intermediate **7a** with aryl boronic acid pinacol esters, followed by acid catalyzed deprotection, when needed, afforded the desired compounds **10**, **14,** and **16**. Stille coupling of **7a-i** with 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol afforded analogs **12, 23, 25, 27, 30, 32, 33, 34,** and **37**. Alternatively the reactivity of the Stille coupling partners was reversed with the conversion of bromide **7a** to stannane **8a**. Aryl bromides were reacted with **8a** under Stille coupling conditions, followed by acid catalyzed deprotection, when needed, to provide compounds **19** and **21**.

Scheme 1. Synthesis of 4,6-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-ones (RE1).



Reagents and conditions: (**a**) R_1-NH_2, acetonitrile, DIPEA, rt or 40-70 °C, 0.5-16 h, yield 24-79%; (**b**) R_2-boronate ester, sodium carbonate in water, $PdCl_2(dppf)$-CH_2Cl_2, dioxane or DMF, microwave 120 °C, 15-30 min, yield 25-74%; (**c**) HCl (4N in dioxane or 6N aqueous), ethanol or dioxane, rt or 45-90 °C, 1-12 h, yield 22-39% (**d**) 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol, $PdCl_2(dppf)$-CH_2Cl_2 or $Pd(PPh_3)_2Cl_2$, DMF, 110-140 °C, 1-2 h, yield 9-66%; (**e**) hexamethylditin, $Pd(PPh_3)_4$, dioxane, 100 °C, 5 h, yield 66%; (**f**) R_2-bromide, $PdCl_2(dppf)$-CH_2Cl_2or $Pd_2(dba)_3$ and P(o-tol)$_3$ and triethylamine, DMF, 100-120 °C, 1-4 h, yield 15-74%.

Scheme 2. Synthesis of 1,7-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-ones (RE2).

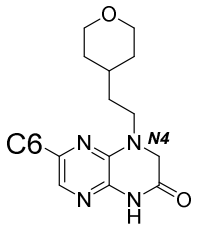


Reagents and conditions: (**a**) R_1-NH_2, DMSO and/or DIPEA, 100-150 °C, 1-48 h, yield 43-74%; (**b**) acetic acid or TFA in methanol, 90-120 °C, 2-24 h, 33-76%; (**c**) R_2-boronate ester, sodium carbonate in water, $PdCl_2(dppf)$-CH_2Cl_2 or $Pd(PPh_3)_2Cl_2$, dioxane, 130-150 °C, 1-4 h, yield 8-44%; (**d**) HCl (4N in dioxane or 6N aqueous), ethanol, 50-100 °C, 0.2-1 h, yield 39-86% (**e**) 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol, $PdCl_2(dppf)$-CH_2Cl_2 or $Pd(dtbpf)Cl_2$ or $Pd(PPh_3)_2Cl_2$, DMF, 110-140 °C, 0.3-2 h, yield 14-68%; (**f**) hexamethylditin, $Pd(PPh_3)_4$, dioxane, 110 °C, 1 h, yield 55%; (**g**) R_2-bromide, $Pd(dtbpf)Cl_2$ or $Pd_2(dba)_3$ and $P(o\text{-}tol)_3$ and triethylamine, DMF, 120 °C, 1 h, yield 33-55%.

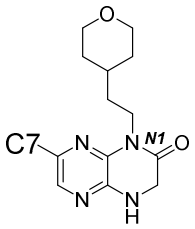
Results and Discussion

We first examined whether the SAR requirements of the C6/C7 position for the ring expansion series would translate as compared to the original imidazo-pyrazinone series. With the N1/N4 ethylene-tetrahydropyran constant, we found the SAR for mTOR kinase potency tracked from the original series, with benzylic alcohols, aryl/heteroaryl triazoles and 6,5-fused heterocycles each maintaining potency in both isomers of the ring expansion series (Table 1).

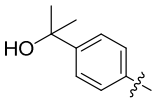
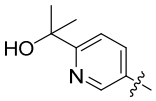
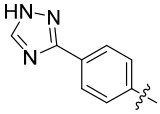
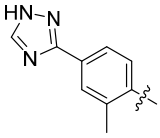
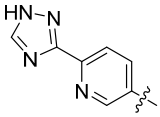
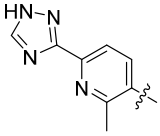
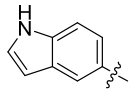
Table 1. SAR of mTOR Kinase and PI3Kα for compounds **9-21**.



RE1



RE2

Analog	C6/C7	Core	mTOR Kinase IC_{50} (μM)[a]	PI3K IC_{50} (μM)[b]
9		RE1	0.066 ± 0.032	NT[c]
10		RE2	0.025 (n=1)	NT
11		RE1	0.103 ± 0.031	NT
12		RE2	0.033 ± 0.008	NT
13		RE1	0.008 ± 0.007	0.53
14		RE2	0.004 ± 0.001	0.10
15		RE1	0.004 ± 0.001	0.20
16		RE2	0.005 (n=1)	0.12
17		RE1	0.018 ± 0.005	1.6
18		RE1	0.090 ± 0.015	2.3
19		RE2	0.014 ± 0.002	0.51
20		RE1	0.122 ± 0.030	NT
21		RE2	0.072 ± 0.028	NT

[a]mean± SEM, [b]n=2 or more, [c]NT = not tested

As observed in the imidazo-pyrazinone series, the triazole phenyl substituted analogs proved to be among the most potent, with single digit nanomolar IC_{50} values against mTOR kinase. As we evaluated the PK properties of the ring expansion series, we first looked at the more potent triazole-phenyl compounds. While the RE2 analog of the triazole-phenyl (**14**) was not tested in PK studies due to poor in vitro metabolic stability (RatS9 48% remaining at 60 min), the RE1 triazole-phenyl analog (**13)** showed an improvement in IV clearance properties as compared to CC214-1, but unfortunately, showed no exposure upon oral dosing (Table 2).

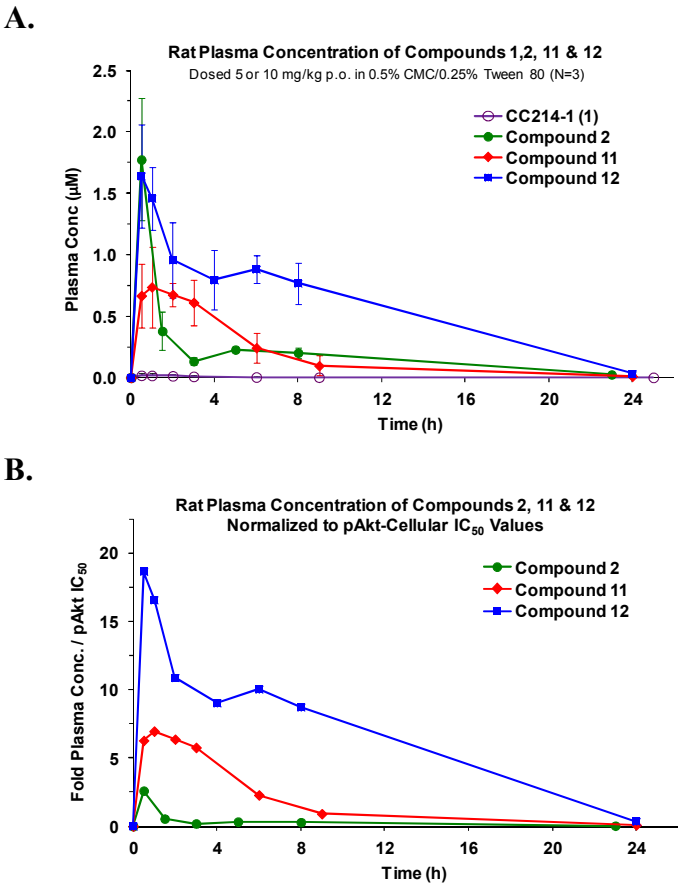


Figure 2. (**A**) Rat PK profiles of **1, 13, 2, 11** and **12** show the ring-expanded analogs of **2** provided improved oral exposure. (**B**) Potency normalized exposures further illustrates benefit of new series.

Table 2. Rat PK parameters for compounds **CC214-1 (1), 2, 11, 12** and **13.**

Rat PK Parameter	Mean (± SD) Value				
	CC214-1	**13**	**2**	**11**	**12**
po Dose [a]	10 mg/kg	10 mg/kg	5 mg/kg	5 mg/kg	5 mg/kg
po C_{max} (μM)	0.024±0.019	BLQ[b]	1.8 ± 0. 5	1.5 ± 0.7	1.7 ± 0.4
po t_{max} (hr)	1.0 ± 0.7		0.5 ± 0	1.5 ± 0	0.6 ± 0.3
po $AUC_{(0-\infty)}$ (μM·hr)	0.11 ±0.08		4.8 ± 0.8	11 ± 6	14 ± 2.1
po F(%)	0.85 ± 0.66		43 ± 9	~100	58 ± 18
iv CL (mL/min/kg)[c]	32 ± 9	13.8 ± 5.7	15 ± 2	23 ± 2	9.0 ± 2.1
Rat S9 Stability[d]	93 ± 12	61 ± 8	91± 2	95 ± 4	73 ± 3

[a]formulated as a suspension in aqueous 0.5% CMC/0.25% Tween-80, [b]BLQ=below limit of quantitation, limit of quantitation 0.00213 μM, [c]dose 2 mg/kg, [d](% remaining at 60 min)

We next evaluated the direct comparisons of the matched ring expansion analogs to the compound which gave the best PK profile in the original imidazo[4,5-b]pyrazin-2-one series, **2**. Both analogs in the ring expansion series, **11** and **12,** provided equivalent or improved PK profiles as compared to the imidazo[4,5-b]pyrazin-2-one counterpart, **2** (Figure 2A and Table 2). In addition to the favorable PK profiles, these ring expansion analogs also showed improved potency and cellular efficacy as compared to the previous series. The potency normalized exposures, shown in Figure 2B, further illustrate the improved potential of the new cores, suggesting the ring expansion series could be optimized for in vivo testing.

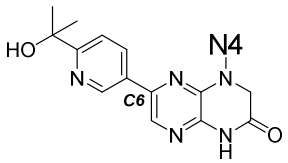
Given the improved PK profiles of the ring expansion series substituted with the pyridinyl-isopropyl alcohol aryl group, we sought to explore the SAR requirements of the N4 and N1 position in the RE1 and RE2 series, respectively. The SAR on mTOR kinase in the previous series suggested favorable interactions for an H-bond or polar substituent in the binding

pocket of the N1/N4 substituent. In addition to potential potency benefits, the improvement in drug-like properties, such as LogD, led us to primarily focus on ether or alcohol substituents (Table 3).

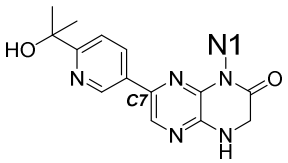
Comparison of the directly connected THP (**25**) with **27** and **12** shows that extension of the THP into the binding pocket results in an improvement in potency. The shorter but slightly more flexible methoxyethyl analog **23** has similar potencies as compared to the directly connected THP analog, **25**. Comparison of analog **25** with the substituted cyclohexyl analogs **34** and **37**, or **24** with **36**, also suggests extension of the polar group further into the binding pocket generally affords improved potencies. The comparison of *cis/trans* pairs **30/32**, **33/34**, or **35/36** suggests that proper positioning of the polar group in the binding pocket plays a role in potency. Intriguingly in the directly connected hydroxy- or methoxy-cyclohexyl analogs, the *trans*-isomer demonstrated a potency benefit relative to the *cis*, while the *cis*-isomer proved more potent when the group was extended by a methylene further into the binding pocket (**30** vs **32**). This *cis/trans* preferences shown here held across multiple other subseries in the course of the program, although in the absence of a protein co-crystal structure we can only speculate about proper alignment for favorable interactions.

These SAR explorations also showed the RE2 series to be generally more potent than the corresponding RE1 analogs. Both ring expansion series maintained selectivity over PI3K-α, particularly in the pyridyl-isopropanol aryl subseries. Compounds from the C6/C7 aryl-triazole subseries picked up some potency against PI3K (Table 1), but maintained >20-88 fold mTOR kinase selectivity. In the pyridyl-isopropanol aryl subseries analogs maintained >20-400 fold selectivity over PI3K, and no analog demonstrated a PI3K IC_{50} value below 1 μM (Table 3).

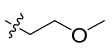
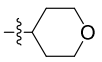
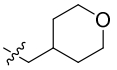
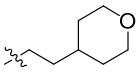
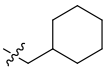
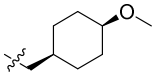
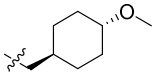
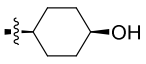
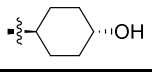
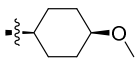
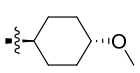
Table 3. SAR of mTOR Kinase and PI3Kα for compounds **11, 12** and **22-37**.



RE1



RE2

Analog	N4/N1	Core	mTOR Kinase IC_{50} (μM)[a]	PI3K IC_{50} (μM)[b]
22		RE1	0.436 ± 0.111	10.5
23		RE2	0.175 ± 0.013	6.2
24		RE1	0.185 ± 0.025	4.7
25		RE2	0.168 ± 0.122	3.2
26 (CC214-2)[13]		RE1	0.106 ± 0.026	>30
27		RE2	0.046 ± 0.019	3.8
11		RE1	0.103 ± 0.031	NT[c]
12		RE2	0.033 ± 0.008	NT
28		RE1	0.108 ± 0.035	17.7
29		RE1	0.149 ± 0.039	7.6
30		RE2	0.012 ± 0.003	2.5
31		RE1	0.170 ± 0.009	20.2
32		RE2	0.097 ± 0.043	4.7
33		RE2	0.203 ± 0.093	2.8
34		RE2	0.038 ± 0.023	1.9
35		RE1	0.300 ± 0.104	11.1
36		RE1	0.103 ± 0.017	16.4
37		RE2	0.010 ± 0.001	4.0

[a]mean ± SEM, [b]n=2 or more, [c]NT = not tested

With potent and selective inhibitors of mTOR kinase identified, we evaluated the inhibitors' effect on the mTOR pathway in PC-3 prostate cancer cells. Inhibition of pS6 (TORC1) and pAkt(S473) (TORC2) were assessed following 1 hour of compound treatment. We also assessed proliferation as a functional effect resulting from mTOR pathway inhibition in these cells. As expected from targeting the mTOR kinase domain, inhibition of both TOR-complexes was observed and this pathway inhibition lead to anti-proliferative effects. In PC-3 prostate cancer cells a good correlation was demonstrated between the enzymatic mTOR kinase potency and cellular biomarker potency. Potency of both TORC1 and TORC2 inhibition correlated with inhibition of cellular growth in the proliferation assays, suggesting that in this cell line the PI3K/mTOR pathway is driving cell survival (Figure 3 and Sup. Table 1-2).

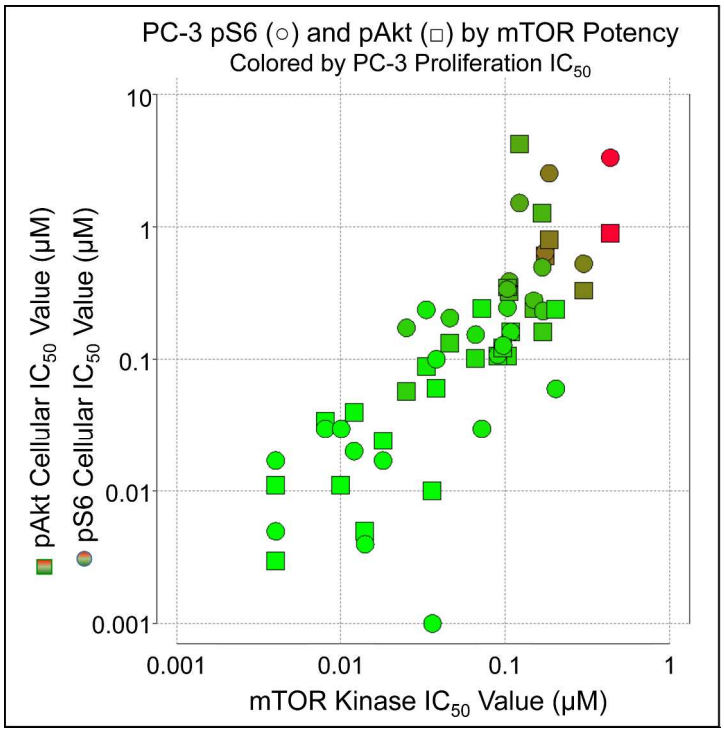


Figure 3. Correlation of mTOR kinase enzyme and cellular biomarker (pS6 and pAkt), colored by proliferation potency (*green* 0.034 to >5 μM *red*). Supplemental tables 1 and 2 provide individual compound data (mean ± SEM).

Having identified a number of compounds in the C6/7-pyridyl-isopropanol aryl ring expansion series with mTOR kinase potency, selectivity and cellular efficacy, we next examined the rodent PK profiles of the analogs to support in vivo efficacy studies. In vitro metabolic stability studies were used to select analogs for in vivo evaluation. Compounds such as **28, 29, 30** and **31** did not meet the stability criteria to support in vivo evaluation (Table 4). The remaining analogs exhibited in vitro stability values of ≥73% remaining after 60 minute incubation with rat liver S9 enzyme fractions. Results of rat oral PK studies demonstrated that most of the analogs in this subseries provided equivalent or improved PK profiles as compared to our initial analogs **11** and **12**. Compounds generally showed good absorption properties, reaching C_{max} values as high as 6.3 μM while providing high AUCs with good duration of exposure, corresponding to oral bioavailability ranging from 43-100% (Table 4).

Based on these results, compounds were selected for single dose PK/PD studies assessing mTOR pathway biomarker inhibition in tumor bearing mice. PC-3 tumor-bearing mice were administered with a single dose of test compound, dosed orally at 100 mg/kg, and plasma and tumor samples were collected at various time points for analysis. Significant inhibition of mTOR pathway markers pS6 and pAktS473 were observed, indicating inhibition of both mTORC1 (pS6) and mTORC2 (pAktS473). Most analogs tested demonstrated full biomarker inhibition in the PC-3 tumor model through 24 hours (Table 5). Over the course of our in vivo studies on, we observed reasonable PK/PD correlations for compounds in this series, including those discussed here. When a compound maintained exposure at sufficient levels to inhibit the target, as estimated from the cellular IC_{50} values, biomarker suppression was observed. Lesser inhibition of both pS6 and pAkt were observed as compound levels decreased in plasma and tumor. We typically observed suppression of ≥80% and ≥60% for pS6 and pAkt, respectively, when tumor

concentrations were >5 fold over the respective cellular IC_{50} values (Sup. Tables 2-4). Because of the intricacies of the PK/PD studies, and to better correlate effects at low compound levels, we conducted single dose biomarker studies using lower doses of **37** (1, 10 or 25 mg/kg). We constructed a refined PK/PD model with the findings and the results have been published elsewhere.[10]

Table 4. In Vitro and In Vivo PK Properties.

Analog	Rat S9 Met. Stability[b]	Rat IV CL (mL/min/kg)[c]	Rat Oral PK Parameter[a]		
			C_{max} (μM)	$AUC_{(0-\infty)}$ (μM·hr)	F(%)
23	89 ± 2	NT[d]	5.6 ± 2.4	29 ± 4.5	NC[e]
26	~100	8.1 ± 0.9	3.3 ± 0.7	28 ± 3	~100
27	83 ± 3	NT	1.6 ± 0.53	9. 9 ± 1.8	NC
11	95 ± 4	23.3 ± 1.9	1.5 ± 0.7	11 ± 6	~100
12	73 ± 3	9.0 ± 2.1	1.7 ± 0.4	14 ± 2.1	58 ± 18
28	16 ± 0.5	NT		NT	
29	39 ± 1	NT		NT	
30	14 ± 1	NT		NT	
31	77 ± 2	NT	0.70 ± 0.23	7.25 ± 2.25	NC
32	55 ± 6	NT		NT	
34	97 ± 6	NT	2.20 ± 1.12	9.23 ± 2.45	NC
35	87 ± 2	NT	2.77 ± 0.77	26.6 ± 7.5	NC
36	94 ± 5	1.7 ± 0.5	6.3 ± 0.9	90 ± 22	70 ± 28
37	81 ± 9	4.9 ± 0.7	2.9 ± 0.9	26 ± 0.7	59 ± 8.4

[a]dose 5 mg/kg in aqueous 0.5% CMC/0.25% Tween-80, mean , [b](% remaining at 60 min), [c]dose 2 mg/kg, [d]NT: not tested, [e]NC: IV study not available.

Table 5. In Vitro Cellular Potency and In Vivo Single Dose Biomarker Inhibition.

	Analog	23	26	27	11	12	31	35	36	37
Cellular pS6 IC_{50}[a]		0.65	0.39	0.2	0.24	0.24	0.23	0.53	0.34	0.03
In Vivo pS6[b]	**2 h**	NT[c]	88	NT	87	71	NT	80	87	NT
	4 h	82	86	93	63	80	80	54	90	91
	8 h	91	88	94	64	88	64	52	85	92
	24 h	91	83	91	0	59	0	70	85	91
Fold Tumor Conc./pS6 Cellular IC_{50}	**2 h**	NT	13.2	NT	6	30.3	NT	6.4	12.9	NT
	4 h	37.6	5.1	27.4	2.7	4	6.8	2.4	12.1	140.3
	8 h	18.2	5.2	9.4	3.5	5.5	4.7	2.6	7.7	31
	24 h	10	7.6	11.3	0.3	4.5	1	3.7	9.3	39.3
Cellular pAkt IC_{50}[a]		0.61	0.32	0.13	0.11	0.09	0.16	0.33	0.35	0.01
In Vivo pAkt[b]	**2 h**	NT	85	NT	74	82	NT	72	75	NT
	4 h	87	73	86	63	63	55	34	62	80
	8 h	84	71	81	67	78	55	59	69	82
	24 h	77	65	80	28	69	0	45	61	86
Fold Tumor Conc./pAkt Cellular IC_{50}	**2 h**	NT	16.0	NT	13.2	80.7	NT	10.2	12.6	NT
	4 h	40.0	6.3	42.2	5.9	10.7	9.8	3.9	11.7	421.0
	8 h	19.4	6.4	14.5	7.6	14.8	6.8	4.2	7.5	93.0
	24 h	10.6	9.3	17.3	0.6	12.1	1.4	5.9	9.0	118.0

[a] μM, SEM for cellular data available in supplemental material, [b]% inhibition, [c]NT=not tested.

Based on both rat PK profiles and the single-dose PK/PD studies, compounds **11, 12, 26, 36** and **37** were selected for evaluation in PC-3 tumor bearing efficacy models. Tumor volumes were determined prior to the initiation of treatment and were considered as the starting volumes. When tumors reached approximately 125 mm^3, mice were randomized and treated once daily

(qd) or twice daily (bid) orally with vehicle or various doses of test compound, at a dose volume of 5 mL/kg. The bid doses were administered with a 10 h separation between the morning and evening doses. Animals were treated for 21 days and the final tumor volume reductions reported here were measured following the final day of dosing. All analogs assessed showed dose- and schedule-dependent inhibition of tumor growth in the PC-3 model. The maximum observed efficacy for each compound, at a tolerated dose, is shown in Table 6. Compound **11** required twice daily dosing to achieve efficacy, a finding that is consistent with the minimal inhibition observed at 24 hours with **11** in the biomarker study. The remaining compounds achieved significant efficacy with once daily dosing. When dosed once daily at 50 mg/kg, analogs **12**, **26** and **36** showed 59%, 84% and 90% tumor volume reductions (TVR= (vehicle - treated / vehicle)x100%), respectively. Compound **37** achieved similar efficacy (87% TVR) at a lower dose of 25 mg/kg. While the PK properties of **26** and **36** are similar to or better than those of **37**, the superior cellular potency of **37** translated to efficacy at lower doses.

Table 6. In Vivo Efficacy Studies in PC-3 Xenograft.[a]

	Maximum Efficacy with Tolerated Dose	
	Dose (mg/kg)	**TVR (%)**
11	100 bid	72
12	50 qd[b]	59
26	50 qd	84
36	50 qd	90
37	25 qd	87

[a]Assay details published previously.[10] [b]Highest dose tested.

The data described herein, along with assessment in additional in vivo models, safety studies and higher species PK informed the selection of **37**, CC-223, for clinical development. CC-223 demonstrates the excellent kinase selectivity achieved in this series. When screened against a commercial panel of >240 kinases, other than mTOR kinase, only 3 were targets were inhibited >80% at 10 μM. Follow-up studies showed only two of these were inhibited with IC_{50} values below 1 μM; Flt4 (0.651 μM) and cFMS (0.028 μM). The kinsase selectivity was confirmed in a cellular assessment, where no kinase other than mTOR was identified when cells were treated with 1 μM of **37** and assayed for kinase activity in ActivX KiNavtiv™ profiling.[10] When tested at concentrations up to 10 μM, CC-223 does not inhibit CYP enzymes and when screened in a single point assay at 10 μM against a Cerep receptor and enzyme panel no target was inhibited >60%. CC-223 was negative in AMES mutagenicity and in vitro micronucleus tests and has a hERG (human ether-a-go-go-related gene) ion channel IC_{50} value of 33 μM. The expanded in vitro and in vivo characterization of the mTOR kinase activity of CC-223 has been reported.[10]

Conclusion

In summary we have described the SAR and optimization of a series of 4,6- *or* 1,7-disubstituted-3,4-dihydropyrazino[2,3-b]pyrazine-2(1H)-one based mTOR kinase inhibitors. The series maintains mTOR kinase potency with exquisite kinase selectivity including the related PI3Kα. Focused investigation in the C6/7-(6-(2-hydroxypropan-2-yl)pyridin-3-yl)-substituted analogs allowed for the identification of a number of compounds with oral PK properties suitable for in vivo studies. This work ultimately led to the identification of CC-223, with superior physicochemical and pharmacokinetic properties, excellent kinase selectivity, demonstrated efficacy across multiple in vivo solid tumor models with oral dosing, in addition to favorable in

vivo and in vitro safety profiles, suitable for clinical development. The results of the Phase I clinical investigations with CC-223 have been reported.[11]

Materials and Methods

Chemistry Compounds were named using ChemDraw Ultra. All materials were obtained from commercial sources and used without further purification, unless otherwise noted. Chromatography solvents were HPLC grade and used as purchased. All air-sensitive reactions were carried out under a positive pressure of an inert nitrogen atmosphere. Reported yields are unoptimized. ^{1}H NMR spectra were obtained on a Varian 400 MHz spectrometer with tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are reported in ppm downfield of TMS and coupling constants (*J*) are given in Hz. Thin Layer Chromatography (TLC) analysis was performed on Whatman thin layer plates. LCMS analysis was performed on a PE Sciex ESI MS or Agilent 1100 MS. Preparative reverse phase HPLC was performed on a Shimadzu system equipped with a Phenomenex 15 micron C18 column (250 x 50 mm). Semi-preparative reverse phase HPLC was performed on a Shimadzu system equipped with a Phenomenex 15 micron C18 column (250 x 10 mm). The purity of final tested compounds was typically determined to be $\geq$ 95% by HPLC conducted on an Agilent 1100 system using a reverse phase C18 column and diode array detector (compounds **20**, **21** and **32** were tested at 94% purity). Compounds were analyzed for purity by one of two methods: (A) Gradient (0-75% acetonitrile + 0.1% formic acid in water + 0.1% formic, over 7 min, followed by 75% acetonitrile + 0.1% formic acid for 2 min); Flow Rate 1 mL/min, Column Phenomenex Gemini-NX 5u C18 110A (50x4.60mm); (B) Gradient (0-75% acetonitrile + 0.1% formic acid in water + 0.1% formic, over 20 min, followed by 75% acetonitrile + 0.1% formic acid for 5 min); Flow Rate 1 mL/min, Column Phenomenex Gemini-NX 5u C18 110A (250x4.60mm). Melting points

were determined on either a manual Electrothermal Mel-Temp® or Stanford Research Systems' OptiMelt System and are uncorrected. Elemental analysis was performed at Robertson Microlit Laboratories, Ledgewood, New Jersey.

Synthetic procedures for all intermediates **4a-i, 5a, 7a-i** and **8a** are included in the supplemental material. All assay procedures have been published previously.[10]

General Procedure A (9, 10, 13-THP, 14-THP, 15-THP, 16-THP). Substrate, boronate ester and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane or dichlorobis(triphenylphosphine)palladium(II) were combined in dioxane or DMF. Sodium carbonate in water was then added. The solution was then heated at 120 °C in a microwave reactor for 15-30 min or on an oil bath at 130-150 °C for 1-4 h. The cooled reaction solutions were filtered through Celite and the filter cake was washed with ethyl acetate. The filtrate and ethyl acetate wash were combined and solvent was removed under reduced pressure. The resulting products were purified using silica gel chromatography or reverse phase HPLC.

General Procedure B (13-19). Substrate was dissolved in ethanol or dioxane and treated with HCl (4N in dioxane or 6N aqueous). The reaction mixtures were stirred at rt or 45-110 °C for 0.2-12 h. The solutions were concentrated under reduced pressure and products were purified using reverse phase HPLC.

General Procedure C (11, 12, 17-THP, 18-THP, 19-THP, 20-37). Aryl bromide, stannane and palladium catalyst were combined in triethylamine and/or DMF. The solution was then heated at 100-140 °C for 0.3-4 h. The reaction mixtures were partitioned between organic solvent (ethyl acetate or methylene chloride) and water. The organic solvents were dried and concentrated under reduced pressure. Products were purified using silica gel chromatography or reverse phase HPLC.

6-(4-(2-Hydroxypropan-2-yl)phenyl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (9). **4a** (0.250 g, 0.733 mmol) and 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-2-ol (0.192 g, 0.733 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.030g, 0.037 mmol) were reacted according to Procedure A to give **9** (0.074 g, 0.19 mmol, 25% yield, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.98 (s, 1H), 7.89 (d, *J=8.39* Hz, 2H), 7.53 (d, *J=8.39* Hz, 1H), 5.04 (s, 1H), 4.16 (s,1H), 3.82 (dd, *J=11.1, 2.39* Hz, 2H), 3.61 (t, *J=7.59,* Hz 2H), 3.25 (t, *J=9.59* Hz, 3H), 1.70 (s, 1H), 1.66 (s, 1H), 1.58 (m, 3H), 1.44 (s, 6H), 1.25 (m, 2H); MS (ESI) *m/z* 397.2 $[M+1]^+$; mp 210-212 °C.

7-(4-(2-Hydroxypropan-2-yl)phenyl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (10). **7a** (500.0 mg, 1.465 mmol), 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-2-ol (461 mg, 1.758 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (53.6 mg, 0.073 mmol) were reacted according to Procedure A to provide the title compound (94.7 mg, 0.239 mmol, 16.3% yield, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1 H), 7.83 - 7.87 (m, 2 H), 7.50 - 7.54 (m, 3 H), 5.03 (s, 1 H), 4.18 (d, *J*=1.6 Hz, 2 H), 4.07 - 4.13 (m, 3 H), 3.83 (dd, *J*=10.3, 3.3 Hz, 3 H), 3.22 - 3.31 (m, 2 H), 1.69 (d, *J*=12.1 Hz, 3 H), 1.53 - 1.62 (m, 4 H), 1.44 (s, 6 H), 1.16 - 1.29 (m, 2 H); MS (ESI) *m/z* 397.2 $[M+1]^+$; mp 198-199 °C.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (11). **4a** (10.0 g, 29.3 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (9.67 g, 32.2 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (4.79 g, 5.86 mmol) were reacted according to Procedure C to give the title compound (3.95 g, 9.94

mmol, 34 % yield, HPLC purity (A) >98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1 H), 9.06 (d, *J*=1.95 Hz, 1 H), 8.27 (dd, *J*=8.59, 2.34 Hz, 1 H), 8.06 (s, 1 H), 7.72 (d, *J*=8.20 Hz, 1 H), 5.27 (s, 1 H), 4.19 (s, 2 H), 3.81 (dd, *J*=11.52, 2.54 Hz, 2 H), 3.62 (t, *J*=7.03 Hz, 2 H), 3.25 (td, *J*=11.62, 1.76 Hz, 2 H), 1.68 (d, *J*=12.49 Hz, 2 H), 1.51 - 1.63 (m, 3 H), 1.46 (s, 6 H), 1.16 - 1.29 (m, 2 H); MS (ESI) *m/z* 398.1 $[M+1]^+$; mp 239-241 °C; Anal. ($C_{21}H_{27}N_5O_3$-0.4H_2O) Calc. C: 62.34, H: 6.92, N: 17.31; Found C: 62.47, H: 7.07, N: 17.45; KF = 1.80%.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (12). 2-(5-(Trimethylstannyl)pyridin-2-yl)propan-2-ol (2.80 g, 9.35 mmol), **7a** (2.90 g, 8.50 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.69 g, 0.85 mmol) were reacted according to Procedure C to give the title compound (2.15 g, 2.60 mmol, 68% yield, HPLC purity (A) >98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J*=2.3 Hz, 1 H), 8.27 (s, 1 H), 8.23 (dd, *J*=8.2, 2.3 Hz, 1 H), 7.71 (d, *J*=8.2 Hz, 1 H), 7.68 (s, 1 H), 5.25 (s, 1 H), 4.20 (s, 2 H), 4.10 (t, *J*=6.8 Hz, 2 H), 3.82 (dd, *J*=11.3, 2.7 Hz, 2 H), 3.26 (t, *J*=10.7 Hz, 2 H), 1.69 (d, *J*=12.5 Hz, 2 H), 1.57 (t, *J*=5.3 Hz, 3 H), 1.46 (s, 6 H), 1.09 - 1.31 (m, 2 H); MS (ESI) *m/z* 398.4 $[M+1]^+$; mp 158-160 °C; Anal. ($C_{21}H_{27}N_5O_3$-0.1H_2O) Calc. C: 62.46, H: 6.69, N: 17.43; Found C: 62.81, H: 6.69, N: 17.43; KF = 0.48%.

6-(4-(4H-1,2,4-Triazol-3-yl)phenyl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (13). **4a** (0.500 g, 1.46 mmol), 1-(tetrahydro-2H-pyran-2-yl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-1,2,4-triazole (0.677 g, 1.905 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.060 g, 0.073 mmol) were reacted according to Procedure A to give **13-THP** (0.347 g, 0.710 mmol, 48 % yield). MS (ESI) *m/z* 406.2 $[M+1]^+$. Procedure B gave **13** (0.135 g,

0.330 mmol, 22 % yield, HPLC purity (A) >97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.64 (s, 1H), 8.08 (s, 5H), 4.19 (s, 2H), 3.82 (dd, *J*= 2.39, 11.19 Hz, 2H), 3.64 (t, *J*=7.19, 2H), 3.26 (t, *J*=9.99 Hz, 2H), 1.70 (d, *J*=13.99 Hz, 2H), 1.59 (m, 3H), 1.25 (m, 2H); MS (ESI) *m/z* 406.2 $[M+1]^+$; mp 301-303 °C.

7-(4-(1H-1,2,4-Triazol-5-yl)phenyl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (14). **7a** (0.5 g, 1.465 mmol), 1-(tetrahydro-2H-pyran-2-yl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-1,2,4-triazole (0.573 g, 1.612 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.060 g, 0.073 mmol) were reacted according to Procedure A followed by Procedure B to give the title compound (0.050 g, 0.123 mmol, 8.4 % yield over two steps, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (br. s., 1 H), 8.29 (br. s., 4 H), 8.07 (br. s., 4 H), 7.65 (br. s., 1 H), 4.21 (s, 2 H), 4.12 (t, *J*=7.0 Hz, 2 H), 3.84 (dd, *J*=11.3, 2.7 Hz, 2 H), 3.28 (td, *J*=11.7, 2.0 Hz, 2 H), 1.71 (d, *J*=12.9 Hz, 2 H), 1.59 (t, *J*=5.7 Hz, 3 H), 1.13 - 1.32 (m, 2 H); MS (ESI) *m/z* 406.0 $[M+1]^+$; mp 237-239 °C.

6-(2-Methyl-1-(1H-1,2,4-triazol-3-yl)phenyl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (15). **4a** (0.212 g, 0.621 mmol), 3-(3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazole (0.250 g, 0.677 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.023 g, 0.028 mmol) were reacted according to Procedure A to give **(15-THP)** (0.21 g, 0.42 mmol, 74 % yield). MS (ESI) *m/z* 504.5 $[M+1]^+$. Procedure B gave **15** (0.066 g, 0.16 mmol, 35 % yield, HPLC purity (A) >97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 4 H), 8.64 (s, 2 H), 7.85 - 7.97 (m, 7 H), 7.57 - 7.64 (m, 3 H), 7.49 (d, *J*=7.42 Hz, 2

H), 4.18 (s, 8 H), 3.77 (dd, *J*=12.49, 5.08 Hz, 8 H), 3.54 (t, *J*=7.22 Hz, 7 H), 3.22 (s, 8 H), 1.46 - 1.67 (m, 18 H), 1.09 - 1.25 (m, 7 H); MS (ESI) *m/z* 420.3 $[M+1]^+$; mp 262-265 °C.

7-(2-Methyl-4-(1H-1,2,4-triazol-3-yl)phenyl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one hydrochloride (16). 3-(3-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-4-(tetrahydro-2H-pyran-2-yl)-4H-1,2,4-triazole (200 mg, 0.542 mmol), **7a** (185 mg, 0.542 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (22.12 mg, 0.027 mmol) were reacted according to Procedure A followed by Procedure B to give the title compound (0.11 g, 0.26 mmol, 44 % yield over two steps, HPLC purity (A) >96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (br. s., 1 H), 7.97 (s, 1 H), 7.91 (d, *J*=7.8 Hz, 1 H), 7.84 (s, 1 H), 7.55 (d, *J*=7.8 Hz, 1 H), 4.21 (s, 2 H), 3.95 - 4.12 (m, 2 H), 3.78 (dd, *J*=10.9, 2.7 Hz, 2 H), 3.22 (t, *J*=10.7 Hz, 2 H), 2.49 (br. s., 3 H), 1.62 (d, *J*=12.5 Hz, 2 H), 1.44 - 1.58 (m, 3 H), 1.05 - 1.24 (m, 2 H); MS (ESI) *m/z* 420.4 $[M+1]^+$; mp 150 °C (dec).

6-(6-(4H-1,2,4-Triazol-3-yl)pyridin-3-yl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (17). 5-Bromo-2-(1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazol-3-yl)pyridine (0.305 g, 0.987 mmol), **5a** (0.419 g, 0.987 mmol) and tris(dibenzylideneacetone)dipalladium(0) (0.090 g, 0.099 mmol) and tri-*o*-tolylphosphine (0.060 g, 0.197 mmol) were reacted according to Procedure C followed by Procedure B to give the title compound (0.112 g, 0.276 mmol, 28 % yield over two steps, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (br. s., 1H), 9.28 (d, *J* = 1.56 Hz, 1H), 8.49 (dd, *J* = 1.95, 8.20 Hz, 1H), 8.29 (br. s., 1H), 8.20 (s, 1H), 8.16 (d, *J* = 8.59 Hz, 1H), 4.22 (s, 2H), 3.83 (dd, *J* = 2.93, 11.13 Hz, 2H), 3.64 (t, *J* = 7.03 Hz, 2H), 3.21 - 3.31 (m, 2H), 1.69 (d, *J* = 12.49 Hz, 2H), 1.49 - 1.65 (m, 3H), 1.16 - 1.31 (m, 2H); MS (ESI) *m/z* 407.3 $[M+1]^+$.

6-(2-Methyl-6-(4H-1,2,4-triazol-3-yl)pyridin-3-yl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (18). 3-Bromo-2-methyl-6-(1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazol-3-yl)pyridine (0.87 g, 2.69 mmol), **5a** (1.144 g, 2.69 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.271 g, 0.296 mmol) and tri-*o*-tolylphosphine (0.180 g, 0.592 mmol) were reacted according to Procedure C to give **18-THP** (1.0 g, 1.98 mmol, 73.6 % yield). MS (ESI) m/z 505.5 $[M+1]^+$. Procedure B gave **18** (0.326 g, 0.78 mmol, 39 % yield, HPLC purity (A) >95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 7.98 (s, 2H), 7.69 (s, 1H), 4.21 (s, 2H), 3.78 (dd, *J* = 2.93, 11.13 Hz, 2H), 3.49 - 3.61 (m, 2H), 3.22 (td, *J* = 1.76, 11.62 Hz, 2H), 2.69 (s, 3H), 1.44 - 1.71 (m, 5H), 1.08 - 1.27 (m, 2H); MS (ESI) m/z 421.1 $[M+1]^+$; mp 200-201 °C.

7-(2-Methyl-6-(1H-1,2,4-triazol-3-yl)pyridin-3-yl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (19). 3-Bromo-2-methyl-6-(1-(tetrahydro-2H-pyran-2-yl)-1H-1,2,4-triazol-3-yl)pyridine (0.38 g, 1.18 mmol), **8a** (0.5 g, 1.18 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.015 g, 0.024 mmol) were reacted according to Procedure C to give **19-THP** (0.33 g, 0.65 mmol, 55 % yield). MS (ESI) m/z 505.5 $[M+1]^+$. Procedure B gave **19** (0.51 g, 1.19 mmol, 86 % yield, HPLC purity (B) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.96 - 8.04 (m, 2H), 7.89 - 7.94 (m, 1H), 7.66 - 7.77 (m, 1H), 4.22 (s, 2H), 4.04 (t, *J* = 7.61 Hz, 2H), 3.78 (dd, *J* = 2.93, 10.35 Hz, 2H), 3.17 - 3.28 (m, 2H), 2.62 - 2.77 (m, 3H), 1.63 (d, *J* = 14.84 Hz, 2H), 1.54 (t, *J* = 5.86 Hz, 3H), 1.10 - 1.24 (m, 2H); MS (ESI) m/z 421.1 $[M+1]^+$; mp 168-170 °C; Anal. ($C_{21}H_{24}N_8O_2$-0.4 H_2O) Calc. C: 58.98, H: 5.84, N: 26.20; Found C: 58.88, H: 5.83, N: 25.98, KF = 1.72%.

6-(1H-Indol-5-yl)-4-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (20). **5a** (0.700 g, 1.647 mmol), 5-bromo-1H-indole (0.339 g, 1.729 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.120 g, 0.165 mmol) were reacted according to Procedure C to give the title compound (0.092 g, 0.244 mmol, 14.80 % yield, HPLC purity (A) 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 2H), 8.18 (s, 1H), 8.00 (s, 1H), 7.74 (dd, *J* = 1.95, 8.59 Hz, 1H), 7.44 (d, *J* = 8.59 Hz, 1H), 7.37 (t, *J* = 2.54 Hz, 1H), 6.47 (t, *J* = 1.95 Hz, 1H), 4.16 (s, 2H), 3.83 (dd, *J* = 2.73, 11.32 Hz, 2H), 3.65 (t, *J* = 7.22 Hz, 2H), 3.27 (td, *J* = 1.76, 11.62 Hz, 2H), 1.72 (d, *J* = 12.89 Hz, 2H), 1.54 - 1.65 (m, 3H), 1.20 - 1.32 (m, 2H); MS (ESI) *m/z* 378.1 $[M+1]^+$; mp 272-274 °C.

7-(1H-Indol-5-yl)-1-(2-(tetrahydro-2H-pyran-4-yl)ethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (21). **8a** (0.750 g, 1.764 mmol), 5-bromo-1H-indole (0.363 g, 1.852 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.162 g, 0.176 mmol) and tri-*o*-tolylphosphine (0.107 g, 0.353 mmol) were reacted according to Procedure C to give the title compound (0.222 g, 0.588 mmol, 33.3 % yield, HPLC purity (A) 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (br. s., 1H), 8.19 (s, 1H), 8.13 (s, 1H), 7.70 (dd, *J* = 1.56, 8.59 Hz, 1H), 7.44 (d, *J* = 8.59 Hz, 1H), 7.36 (t, *J* = 2.73 Hz, 1H), 7.34 (s, 1H), 6.45 (br. s., 1H), 4.10 - 4.18 (m, 4H), 3.83 (dd, *J* = 2.54, 11.52 Hz, 2H), 3.28 (td, *J* = 1.56, 11.52 Hz, 2H), 1.72 (d, *J* = 12.89 Hz, 2H), 1.53 - 1.64 (m, 3H), 1.20 - 1.31 (m, 2H); MS (ESI) *m/z* 378.2 $[M+1]^+$; mp = 268-270 °C.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(2-methoxyethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (22). **4b** (0.251, 0.836 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (0.251 g, 0.836 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.114 g, 0.139 mmol) were reacted according to Procedure C to

give the title compound (0.055 g, 0.16 mmol, 23 % yield, HPLC purity (A) 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.34 (s, 1 H), 9.06 (d, *J*=1.56 Hz, 1 H), 8.28 (dd, *J*=8.39, 2.15 Hz, 1 H), 8.07 (s, 1 H), 7.71 (d, *J*=8.98 Hz, 1 H), 5.27 (s, 1 H), 4.26 (s, 2 H), 3.77 (m, *J*=5.08 Hz, 2 H), 3.66 (t, *J*=5.86 Hz, 2 H), 3.29 (s, 3 H), 1.46 (s, 6 H); MS (ESI) *m/z* 344.1 $[M+1]^+$; mp 170-172 °C.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(2-methoxyethyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (23). **7b** (230.0 mg, 0.801 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (240.0 mg, 0.801 mmol) and dichlorobis(triphenylphosphine)palladium(II) (117.0 mg, 0.160 mmol) were reacted according to Procedure C to give the title compound (104.0 mg, 0.303 mmol, 37.8 % yield, HPLC purity (A) 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J*=1.6 Hz, 1 H), 8.27 (s, 1 H), 8.24 (dd, *J*=8.6, 2.3 Hz, 1 H), 7.71 (d, *J*=0.8 Hz, 1 H), 7.69 (s, 1 H), 5.25 (s, 1 H), 4.28 (t, *J*=6.2 Hz, 2 H), 4.20 (d, 2 H), 3.60 (t, *J*=6.2 Hz, 2 H), 3.26 (s, 3 H), 1.46 (s, 6 H); MS (ESI) *m/z* 344.3 $[M+1]^+$; mp 160-161 °C.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(tetrahydro-2H-pyran-4-yl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (24). **4c** (0.338 g, 1.079 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (0.389 g, 1.295 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.176 g, 0.216 mmol) were reacted according to Procedure C to give the title compound (0.085 g, 0.230 mmol, 21.3 % yield, HPLC purity (A) 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.34 (s, 1H), 9.08 (dd, *J* = 0.73, 2.29 Hz, 1H), 8.30 (dd, *J* = 2.34, 8.30 Hz, 1H), 8.09 (s, 1H), 7.72 (dd, *J* = 0.76, 8.32 Hz, 1H), 5.27 (s, 1H), 4.64 - 4.76 (m, 1H), 4.12 (s, 2H), 3.97 (dd, 2H), 3.47 - 3.55 (m, 1H), 1.79 - 1.94 (m, 2H), 1.59 - 1.71 (m, 2H), 1.46 (s, 6H); MS (ESI) *m/z* 370.3 $[M+1]^+$; mp 150-151 °C.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (25). **7c** (250.0 mg, 0.798 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (239 mg, 0.798 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (58.4 mg, 0.080 mmol) were reacted according to Procedure C to give the title compound (42.3 mg, 0.115 mmol, 14.34 % yield, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, *J*=2.3 Hz, 1 H), 8.29 (s, 1 H), 8.26 (dd, *J*=8.2, 2.3 Hz, 1 H), 7.71 (d, *J*=8.6 Hz, 1 H), 7.63 (s, 1 H), 5.26 (br. s., 1 H), 5.10 - 5.20 (m, 1 H), 4.15 (d, *J*=1.6 Hz, 2 H), 4.00 (dd, *J*=10.9, 4.3 Hz, 2 H), 3.40 - 3.49 (m, 2 H), 2.80 (qd, *J*=12.4, 4.3 Hz, 2 H), 1.57 (dd, *J*=12.1, 1.6 Hz, 2 H), 1.47 (s, 6 H); MS (ESI) *m/z* 370.2 $[M+1]^+$.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-((tetrahydro-*2H*-pyran-4-yl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(*1H*)-one (CC214-2, 26). **4d** (35.98 g, 110 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (33.0 g, 110 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (8.05 g, 11.00 mmol) were reacted according to Procedure C to give the title compound (15.08 g, 39.3 mmol, 35.8% yield, HPLC purity (A) >99%). Combined batches were recrystallized from ethanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1 H), 9.07 (d, *J*=1.56 Hz, 1 H), 8.29 (dd, *J*=8.59, 2.34 Hz, 1 H), 8.05 (s, 1 H), 7.72 (d, *J*=8.20 Hz, 1 H), 5.26 (s, 1 H), 4.21 (s, 2 H), 3.83 (d, *J*=2.73 Hz, 2 H), 3.51 (d, *J*=7.42 Hz, 2 H), 3.27 (t, *J*=11.32 Hz, 2 H), 2.09 (br. s., 1 H), 1.61 (d, *J*=11.3 Hz, 2 H), 1.46 (s, 6 H), 1.24 - 1.38 (m, 2 H); MS (ESI) *m/z* 384.2 $[M+1]^+$; mp 268 - 269 °C; Anal. ($C_{20}H_{25}N_5O_3$) Calc. C: 62.65, H: 6.57, N: 18.26; Found C: 62.25, H: 6.55, N: 18.19.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (27). **7d** (700.0 mg, 2.140 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (642.0 mg, 2.140 mmol) and dichlorobis(triphenylphosphine)palladium(II) (313 mg, 0.428 mmol) were reacted according to Procedure C to give the title compound (131.2 mg, 0.342 mmol, 15.99 % yield, HPLC purity (A) 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (d, *J*=1.6 Hz, 1 H), 8.27 (s, 1 H), 8.25 (dd, *J*=8.2, 2.3 Hz, 1 H), 7.72 (d, *J*=0.8 Hz, 1 H), 7.70 (s, 1 H), 5.24 (s, 1 H), 4.21 (d, *J*=1.6 Hz, 2 H), 4.01 (d, *J*=7.4 Hz, 2 H), 3.83 (dd, 2 H), 3.18 - 3.26 (m, 2 H), 2.01 - 2.13 (m, 1 H), 1.52 - 1.60 (m, 2 H), 1.46 (s, 6 H), 1.25 - 1.37 (m, 2 H); MS (ESI) *m/z* 384.3 $[M+1]^+$.

4-(Cyclohexylmethyl)-6-(6-(2-hydroxypropan-2-yl)pyridin-3-yl)-3,4-dihydropyrazino[2,3-*b*]pyrazin-2(1*H*)-one (28). **4e** (0.410 g, 1.261 mmol), 2-(5-(trimethylstannyl) pyridin-2-yl)propan-2-ol (0.454 g, 1.513 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.206 g, 0.252 mmol) were reacted according to Procedure C to give the title compound (0.045 g, 0.118 mmol, 9.4 % yield, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.05 (dd, *J* = 0.73, 2.29 Hz, 1H), 8.28 (dd, *J* = 2.34, 8.35 Hz, 1H), 8.04 (s, 1H), 7.72 (d, *J* = 8.35 Hz, 1H), 5.26 (s, 1H), 4.19 (s, 2H), 3.46 (d, *J* = 7.08 Hz, 2H), 1.79 - 1.88 (m, 1H), 1.60 - 1.76 (m, 5H), 1.46 (s, 6H), 1.14 - 1.24 (m, 3H), 1.03 (s, 2H); MS (ESI) *m/z* 382.3 $[M+1]^+$.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-((*(trans)*-4-methoxycyclohexyl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (29). **4f** (64.4 mg, 0.181 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (54.4 mg, 0.181 mmol) and dichlorobis(triphenylphosphine)palladium(II) (26.5 mg, 0.036 mmol) were reacted according to Procedure C to give the title compound (13.5 mg, 0.033 mmol, 18.1 % yield, HPLC purity (A)

97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1 H), 9.05 (d, *J*=2.0 Hz, 1 H), 8.27 (dd, *J*=8.4, 2.1 Hz, 1 H), 8.04 (s, 1 H), 7.71 (d, *J*=8.6 Hz, 1 H), 5.26 (s, 1 H), 4.19 (s, 2 H), 3.47 (d, *J*=7.4 Hz, 2 H), 3.20 (s, 3 H), 1.76 - 1.84 (m, 2 H), 1.43 - 1.50 (m, 10 H), 1.29 - 1.44 (m, 4 H); MS (ESI) *m/z* 412.2 $[M+1]^+$; mp 230 - 231 °C.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(((*trans*)-4-methoxycyclohexyl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (30). **7e** (1.20 g, 3.38 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (1.01 g, 3.38 mmol) and dichloro[1,1'-bis(ditert-butylphosphino)ferrocene]palladium (44 mg, 0.068 mmol) were reacted according to Procedure C to give the title compound (0.289 g, 0.728 mmol, 21% yield, HPLC purity (A) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J*=1.56 Hz, 1 H), 8.26 (s, 1 H), 8.23 (dd, *J*=8.20, 2.34 Hz, 1 H), 7.67 - 7.72 (m, 2 H), 5.25 (s, 1 H), 4.21 (d, *J*=1.56 Hz, 2 H), 3.97 (d, *J*=7.03 Hz, 2 H), 3.29 - 3.32 (m, 1 H), 3.19 (s, 3 H), 1.84 - 1.95 (m, 1 H), 1.72 - 1.82 (m, 2 H), 1.46 (s, 6 H), 1.25 - 1.43 (m, 6 H); MS (ESI) *m/z* 412.3 $[M+1]^+$; mp 190 °C.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(((*trans)*-4-methoxycyclohexyl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (31). **4g** (120.0 mg, 0.338 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (101 mg, 0.338 mmol) and dichlorobis(triphenylphosphine)palladium(II) (49.4 mg, 0.068 mmol) were reacted according to Procedure C to give the title compound (24.3 mg, 0.059 mmol, 17.48 % yield, HPLC purity (A) 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1 H), 9.05 (d, *J*=1.6 Hz, 1 H), 8.27 (dd, *J*=8.4, 2.1 Hz, 1 H), 8.04 (s, 1 H), 7.72 (d, *J*=8.2 Hz, 1 H), 5.26 (s, 1 H), 4.19 (s, 2 H), 3.47 (d, *J*=7.0 Hz, 2 H), 3.21 (s, 3 H), 3.04 - 3.13 (m, 1 H), 1.95 - 2.03 (m, 2 H), 1.72 - 1.83 (m, 3 H), 1.46 (s, 6 H), 1.00 - 1.13 (m, 4 H); MS (ESI) *m/z* 412.2 $[M+1]^+$; mp 263 - 264 °C.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(((*trans*)-4-methoxycyclohexyl)methyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (32). **7f** (400 mg, 1.13 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (338 mg, 1.13 mol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (184 mg, 0.23 mmol) were reacted according to Procedure C to give the title compound (114 mg, 0.28 mmol, 25 % yield, HPLC purity (A) 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J*=1.95 Hz, 1 H), 8.27 (s, 1 H), 8.23 (dd, *J*=8.20, 2.34 Hz, 1 H), 7.67 - 7.73 (m, 2 H), 5.25 (s, 1 H), 4.20 (d, *J*=1.56 Hz, 2 H), 3.96 (d, *J*=7.03 Hz, 2 H), 3.20 (s, 3 H), 1.99 (br. s., 2 H), 1.70 (br. s., 3 H), 1.46 (s, 6 H), 0.94 - 1.13 (m, 5 H); MS (ESI) *m/z* 412.8 $[M+1]^+$.

1-(*trans*-4-Hydroxycyclohexyl)-7-(6-(2-hydroxypropan-2-yl)pyridin-3-yl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (33). 2-(5-(Trimethylstannyl)pyridin-2-yl)propan-2-ol (0.234 g, 0.780 mmol), **7g** (0.300 g, 0.709 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.058 g, 0.071 mmol) were reacted according to Procedure C to give the title compound (0.107 g, 0.279 mmol, 39 % yield, HPLC purity (B) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (d, *J* = 2.34 Hz, 1H), 8.50 (dd, *J* = 2.15, 8.39 Hz, 1H), 8.31 (s, 1H), 7.68 (d, *J* = 8.20 Hz, 1H), 7.57 (s, 1H), 5.24 (s, 1H), 4.86 (tt, *J* = 3.47, 12.35 Hz, 1H), 4.53 (s, 1H), 4.13 (d, *J* = 1.56 Hz, 2H), 3.91 (br. s., 1H), 2.93 - 3.12 (m, 2H), 1.81 (d, *J* = 13.67 Hz, 2H), 1.41 - 1.59 (m, 8H), 1.32 (d, *J* = 11.32 Hz, 2H); MS (ESI) *m/z* 384.2 $[M+1]^+$.

1-(*trans*-4-Hydroxycyclohexyl)-7-(6-(2-hydroxypropan-2-yl)pyridin-3-yl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (34). 2-(5-(Trimethylstannyl)pyridin-2-yl)propan-2-ol (0.252 g, 0.841 mmol), **7h** (0.250 g, 0.764 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.062 g,

0.076 mmol) were reacted according to Procedure C to give the title compound (0.098 g, 0.256 mmol, 33 % yield HPLC purity (B) 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 1.95 Hz, 1H), 8.28 (s, 1H), 8.24 (dd, *J* = 2.34, 8.59 Hz, 1H), 7.72 (d, *J* = 8.98 Hz, 1H), 7.61 (s, 1H), 5.25 (s, 1H), 4.87 (tt, *J* = 3.86, 12.15 Hz, 1H), 4.67 (d, *J* = 3.51 Hz, 1H), 4.13 (d, *J* = 1.56 Hz, 2H), 3.44 - 3.56 (m, 1H), 3.17 (s, 1H), 2.53 - 2.69 (m, 1H), 1.95 (d, *J* = 10.15 Hz, 2H), 1.61 (d, *J* = 11.71 Hz, 2H), 1.47 (s, 6H), 1.24 - 1.39 (m, 2H); MS (ESI) *m/z* 384.2 $[M+1]^+$.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(*(trans)*-4-methoxycyclohexyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (35). **4h** (509.2 mg, 1.492 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (448.0 mg, 1.492 mmol) and dichlorobis(triphenylphosphine)palladium(II) (218.0 mg, 0.298 mmol) were reacted according to Procedure C to give the title compound (118.0 mg, 0.297 mmol, 19.89 % yield HPLC purity (A) 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1 H), 9.10 (d, *J*=1.6 Hz, 1 H), 8.31 (dd, *J*=8.2, 2.3 Hz, 1 H), 8.08 (s, 1 H), 7.71 (d, *J*=7.8 Hz, 1 H), 5.27 (s, 1 H), 4.34 - 4.44 (m, 1 H), 4.07 (s, 2 H), 3.42 - 3.46 (m, 1 H), 3.27 (s, 3 H), 1.84 - 2.06 (m, 4 H), 1.48 - 1.61 (m, 4 H), 1.46 (s, 6 H); MS (ESI) *m/z* 398.2 $[M+1]^+$; mp 273 - 274 °C.

6-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-4-(*(trans)*-4-methoxycyclohexyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (36). **4i** (6.0 g, 17.48 mmol), 2-(5-(trimethylstannyl)pyridin-2-yl)propan-2-ol (5.7 g, 18.99 mmol) and dichlorobis(triphenylphosphine)palladium(II) (1.29 g, 1.76 mmol) were reacted according to Procedure C to give the title compound (4.61 g, 11.61 mmol, 66% yield HPLC purity (B) >99%). Combined batches were recrystallized from methanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1 H), 9.07 (d, *J*=1.95 Hz, 1 H), 8.29 (dd, *J*=8.20, 2.34 Hz, 1 H), 8.07 (s, 1 H), 7.73 (d, *J*=8.20 Hz, 1 H), 5.27 (s, 1 H), 4.34 - 4.53 (m, 1 H), 4.08 (s, 2 H), 3.26 (s, 3 H), 3.02 - 3.20 (m, 1 H),

2.12 (d, *J*=12.89 Hz, 2 H), 1.74 (br. s., 2 H), 1.68 (d, *J*=12.49 Hz, 2 H), 1.46 (s, 6 H), 1.16 - 1.39 (m, 2 H); MS (ESI) *m/z* 398.3 $[M+1]^+$; mp 271 - 273 °C; Anal. ($C_{21}H_{27}N_5O_3$-0.5H_2O) Calc. C: 62.05, H: 6.94, N: 17.23; Found C: 61.70, H: 6.95, N: 17.05, KF = 2.17%.

7-(6-(2-Hydroxypropan-2-yl)pyridin-3-yl)-1-(*trans*-4-methoxycyclohexyl)-3,4-dihydropyrazino[2,3-b]pyrazin-2(1H)-one (CC-223, 37). 2-(5-(Trimethylstannyl)pyridin-2-yl)propan-2-ol (9.43 g, 31.4 mmol), **7i** (10.02 g, 29.4 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (2.398 g, 2.94 mmol) were reacted according to Procedure C to give the title compound (4.85 g, 12.20 mmol, 42 % yield). Batches were recrystallized from acetonitrile, (HPLC purity (B) >99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 1.56 Hz, 1H), 8.28 (s, 1H), 8.24 (dd, *J* = 2.34, 8.20 Hz, 1H), 7.74 (d, *J* = 7.81 Hz, 1H), 7.61 (s, 1H), 5.26 (s, 1H), 4.90 (tt, *J* = 3.71, 12.10 Hz, 1H), 4.13 (s, 2H), 3.28 (s, 3H), 3.20 (tt, *J* = 4.00, 10.84 Hz, 1H), 2.58 (qd, *J* = 2.93, 12.82 Hz, 2H), 2.14 (d, *J* = 10.15 Hz, 2H), 1.68 (d, *J* = 10.93 Hz, 2H), 1.47 (s, 6H), 1.17 - 1.35 (m, 2H); MS (ESI) *m/z* 398.3 $[M+1]^+$; mp 196-198 °C; Anal. ($C_{21}H_{27}N_5O_3$) Calc. C: 63.46, H: 6.85, N: 17.62; Found C: 63.52, H: 7.04, N: 17.86; KF = 0.01%.

mTOR and PI3K Kinase Enzyme Assays An HTR-FRET substrate phosphorylation assay was employed for mTOR kinase as described previously.[10] PI3Kα IC_{50} determinations were outsourced to Carna Biosciences (Japan) using the mobility shift assay format. Compounds were assessed against concentrations of ATP at approximately the Km for the assay, with average ATP Km of 15 μM and 50 μM for the mTOR and PI3K assays, respectively.

PC-3 Cellular Assays. PC-3 cells were purchased from and verified by American Tissue Culture Collection and were cultured in growth media as recommended by the vendor. For biomarker studies, cells were treated for 1 h and then assayed for pS6 and pAkt levels using

MesoScale technology. For proliferation experiments, cells were treated with compound and then allowed to grow for 72 h. All data were normalized and represented as a percentage of the DMSO-treated cells. Results were then expressed as IC_{50} values. Full experimental details have been previously published.[10]

In Vivo Studies. All animal studies were performed under protocols approved by Institutional Animal Care and Use Committees. Single dose biomarker and multi-day efficacy studies were performed as previously published.[10]

Supporting Information

Supporting information includes full experimental details for preparation of intermediates, values of cellular data represented graphically in Figure 3 (Supplemental Tables 1 and 2), and plasma and tumor levels from PK/PD studies (Supplemental Table 3, 4 and 5). This material is available free of charge via the Internet at http://pubs.acs.org.

Author Information

*Corresponding Author: Tel: 858-795-4951, dmortens@celgene.com

Present Addresses: Current address information for R. Bisonette, B.Lee, S. Sankar, G. Shevlin and G.Packard may be available from corresponding author upon request.

Notes: The authors declare no competing financial interest. All authors are currently employees of Celgene, except S.Sankar, B.Lee, G.Shevlin, R. Bisonette, and G.Packard, who were employees of Celgene at the time of their contribution to this work.

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Abbreviations: AKT, Protein Kinase B; AUC, area under the curve; cFMS (CSF1R), colony stimulating factor 1 receptor tyrosine kinase; C_{max}, maximum concentration; DIPEA, *N,N*-diisopropylethylamine; FLT4, FMS-related tyrosine kinase; mTOR, Mammalian Target of Rapamycin; mTORC1, mTOR Complex 1; mTORC2, mTOR Complex 2; pAKT, Phosphorylated AKT; pAKT(S473), Phosphorylated AKT at Serine 473; PI3K, Phosphatidylinositol 3-Kinase; PIK3CA, Gene coding 110 kDa catalytic subunit of PI3K alpha; PK/PD, Pharmacokinetic/Pharmacodynamic; pS6RP or pS6, Phosphorylated Ribosomal protein S6; S6RP or S6, Ribosomal protein S6; SEM, Standard error of the mean; TVR, tumor volume reduction.

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TOC GRAPHIC:

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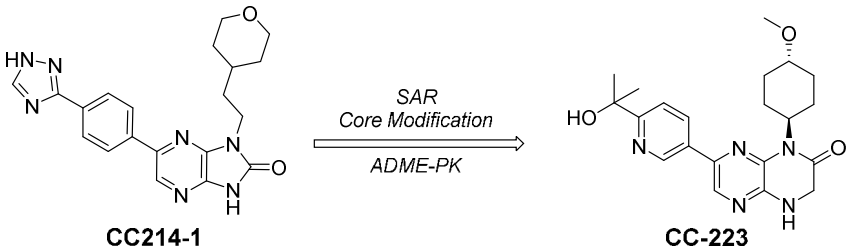

CC214-1
mTOR Kinase IC_{50} = 0.002 μM
PI3Kα IC_{50} = 2.6 μM
PC3 pAkt IC_{50} = 0.018 μM
PO Rat F% = 0.9%

CC-223
mTOR Kinase IC_{50} = 0.016 μM
PI3Kα IC_{50} = 4.0 μM
PC3 pAkt IC_{50} = 0.011 μM
PO Rat F% = 59%

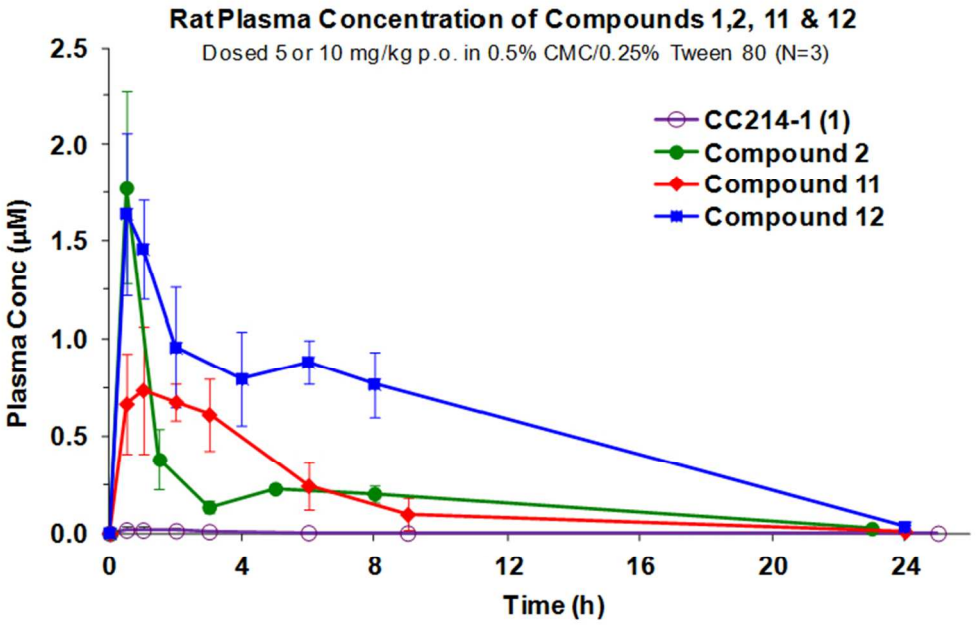

Figure 2. (A) Rat PK profiles of 1, 13, 2, 11 and 12 show the ring-expanded analogs of 2 provided improved oral exposure.
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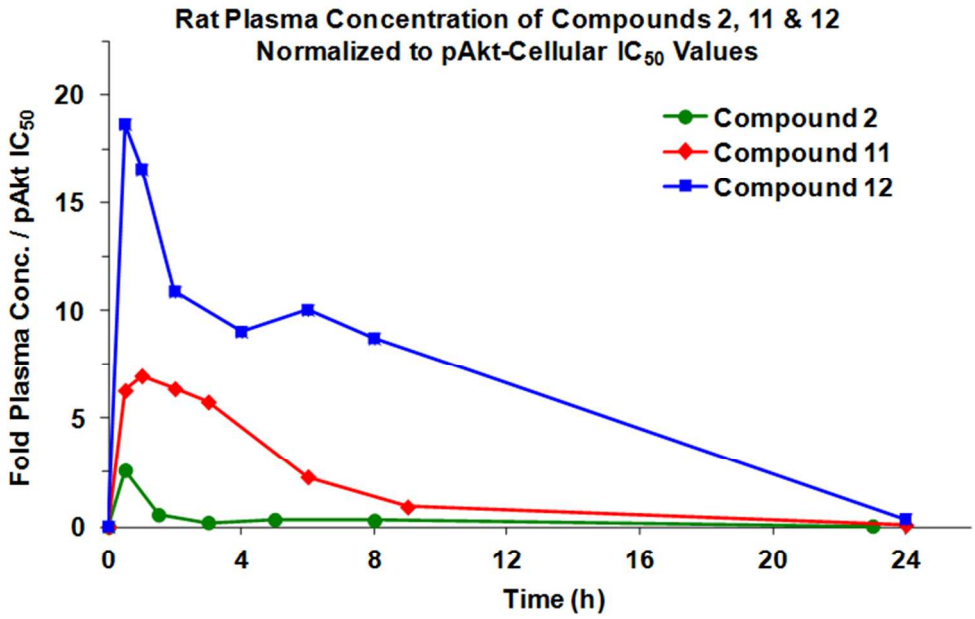

Figure 2. (B) Potency normalized exposures further illustrates benefit of new series.
182x119mm (110 x 110 DPI)

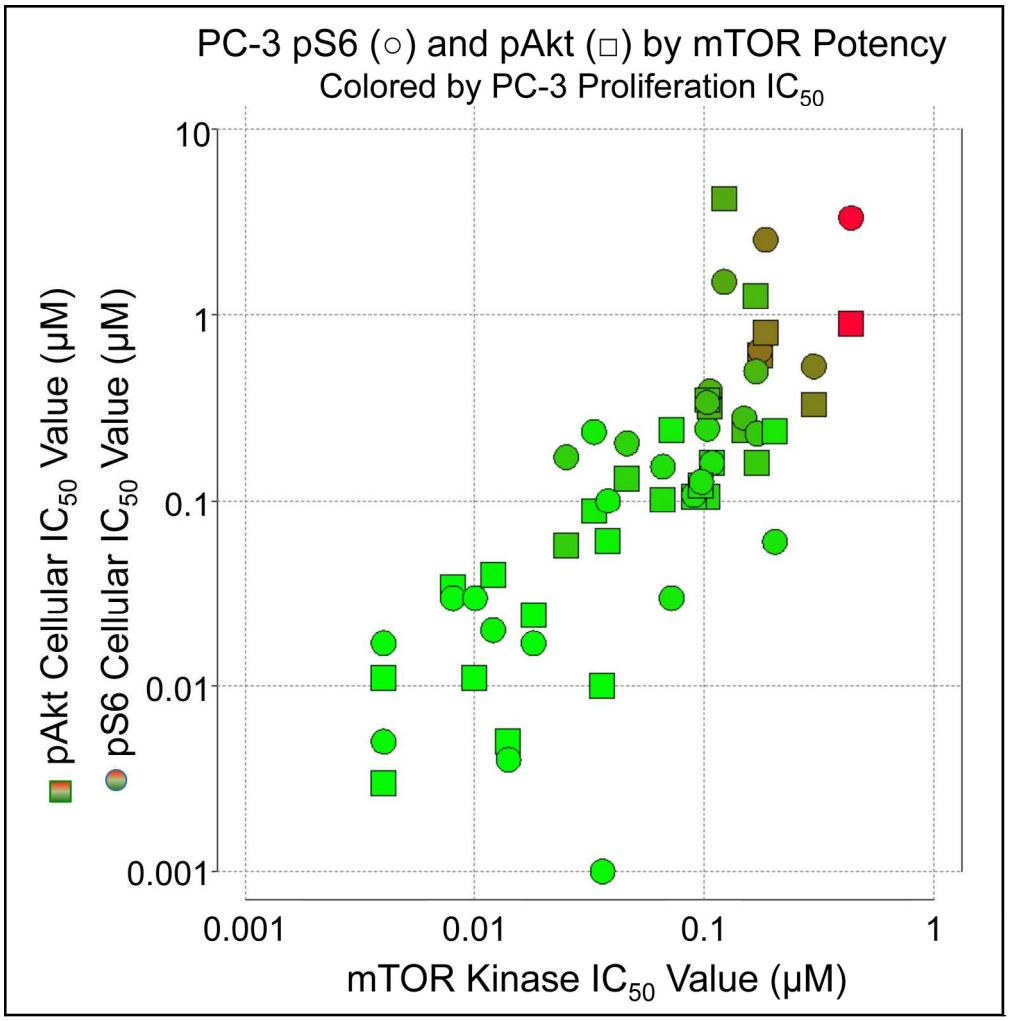

Figure 3. Correlation of mTOR kinase enzyme and cellular biomarker (pS6 and pAkt), colored by proliferation potency (green 0.034 to >5 μM red). Supplemental tables 1 and 2 provide individual compound data (mean ± SEM).
189x190mm (300 x 300 DPI)