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Use of core modification in the discovery of CC214-2, an orally available, selective inhibitor of mTOR kinase

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ABSTRACT

We report here the discovery of a novel series of selective mTOR kinase inhibitors and the identification of CC214-2, a compound with demonstrated anti-tumor activity upon oral dosing in a PC3 prostate cancer xenograft model. A series of 4,6-disubstituted-3,4-dihydropyrazino[2,3-*b*]pyrazine-2(1*H*)-ones were discovered through a core modification of our original compound series. Analogs from this series have excellent mTOR potency and maintain selectivity over the related PI3Kα lipid kinase. Compounds such as CC214-2 were found to block both mTORC1(pS6) and mTORC2(pAktS473) signaling in PC3 cancer cells, in vitro and in vivo.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase member of the phosphatidylinositol 3-kinase-like kinase superfamily. mTOR regulates cell growth, metabolism, proliferation and survival through integration of growth factor signaling with cellular nutritional status and energy use.[1] The mTOR kinase exists within two distinct multiprotein complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2).[2] Both mTORC1 and mTORC2 are critical mediators of the PI3K/AKT pathway, which is frequently mutated in many cancers, leading to hyperactivation of mTOR signaling.[3,4] While rapamycin analogs such as temsirolimus and everolimus, compounds that target only the mTORC1 complex, have shown some clinical activity; it is hypothesized that mTOR kinase inhibitors, blocking both mTORC1 and mTORC2 signaling, will have expanded therapeutic potential.[5] We have previously described the identification and initial SAR exploration of a potent series of 1,6-substituted imidazo[4,5-*b*]pyrazin-2-ones as mTOR kinase inhibitors.[6] Here we describe the use of core modification in the identification of a new series of 4,6-disubstituted-3,4-dihydropyrazino[2,3-*b*]pyrazin-2(1*H*)-one mTOR kinase inhibitors, leading to the identification of CC214-2, an orally available mTOR kinase inhibitor with demonstrated anti-tumor activity in mice with PC3 human prostate cancer tumors.

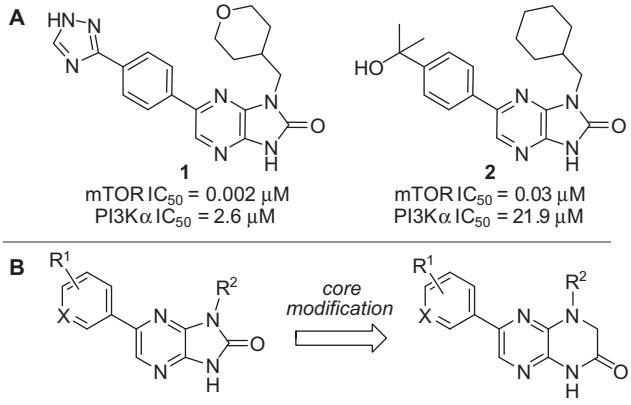

Figure 1. (A) Representative analogs from initial compound series. (B) Core modification of the imidazo[4,5-*b*]pyrazin-2-ones to give 3,4-dihydropyrazino[2,3-*b*]pyrazin-2(1*H*)-ones.

Our original SAR efforts in the imidazo[4,5-*b*]pyrazin-2-one series lead to the identification of analogs such as **1** and **2** (Fig. 1A).

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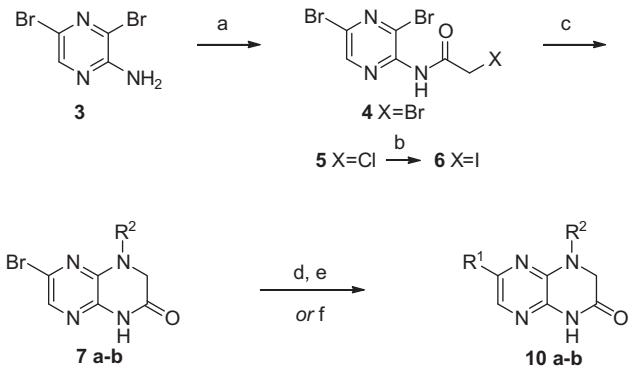


Scheme 1. Reagents and conditions: (a) 2-bromoacetic anhydride or 2-chloroacetic anhydride, acetonitrile, 70 °C, 79–85%; (b) sodium iodide, acetone, 77%; (c) desired amine, acetonitrile, diisopropylethyl amine, rt or 40 °C, 50–79%; (d) 4-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,4-triazol-3-yl)phenyl-boronate ester, sodium carbonate, $PdCl_2(dppf)_2$–CH_2Cl_2, dioxane and water, 120 °C, 48%; (e) 4 N HCl in dioxane, ethanol, rt, 22%; (f) 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol, $PdCl_2(dppf)_2$–CH_2Cl_2, DMF, 110 °C, 29%.

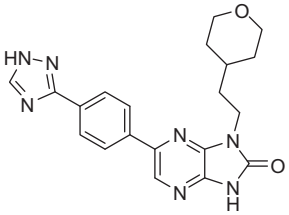
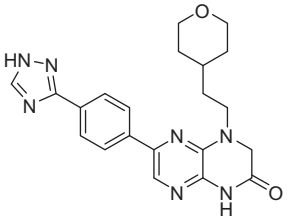
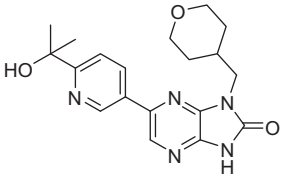
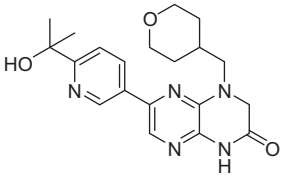
These compounds were potent and selective inhibitors of mTOR kinase and showed inhibition of pathway biomarkers and proliferation in a PC3 prostate cancer cell line.[6]

As we continued our medicinal chemistry exploration of this series, we also sought to examine changes to the imidazo[4,5-*b*]pyrazin-2-one core. Ring-expansion of the imidazo-ring, through insertion of a methylene unit, led to the identification of a new series of potent and selective mTOR kinase inhibitors (Fig. 1B).

Compounds in the ring-expansion series were synthesized through the route outlined in Scheme 1. Treatment of 3,5-dibromopyrazin-2-amine **3** with either bromo- or chloro-acetic anhydride gave **4** or **5**, respectively. Chloro intermediate **5** was converted to the corresponding iodide **6** using sodium iodide. This conversion was necessary as the chloro-intermediate proved unreactive in the subsequent amine addition reactions. Amine additions to **4** or **6** afforded halogen displacement and intramolecular ring closure to give intermediates **7a**–**b**. Compound **10a** (see Table 1) was synthesized through a Suzuki coupling of 4-(1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,4-triazol-3-yl)phenyl-boronic acid pinacol ester to **7a**, followed by acidic deprotection. Stille coupling of 2-(5-(trimethyl-stannyl)pyridin-2-yl)propan-2-ol with **7b** afforded **10b**. Compounds **9a** and **9b** from the imidazo[4,5-*b*]pyrazin-2-one series were synthesized following methods described previously.[6]

Compounds from the newly identified series provided similar potency relative to the parent imidazo[4,5-*b*]pyrazin-2-ones (Table 1). These analogs retained selectivity for mTOR over the related lipid kinase PI3Kα, maintaining 65- to >500-fold selectivity for mTOR. In PC3 prostate cancer cell lines, the compounds proved to be inhibitors of both mTOR complexes as measured by inhibition of pS6 (mTORC1) and pAktS473 (mTORC2), as would be expected for mTOR kinase domain inhibitors. We also observed inhibition

Table 1
mTOR and PI3Kα potency and mTOR pathway biomarker and antiproliferation data in PC3 cancer cells[a]

Analog	Structure	mTOR IC_{50}[b] (μM)	PI3Kα IC_{50}[b] (μM)	PC3 cellular activity		
				pS6 IC_{50}[b] (μM)	pAkt IC_{50}[b] (μM)	Prolif IC_{50}[b] (μM)
9a CC214-1		0.002	1.38	0.040	0.018	0.224
10a		0.008	0.526	0.046	0.034	0.148
9b		0.176	>30[c]	2.41	2.55	4.18[c]
10b CC214-2		0.106	>30[c]	0.386	0.315	1.55

[a] See Supplementary data for assay details.
[b] Average of two or more experiments.
[c] Single experiment.

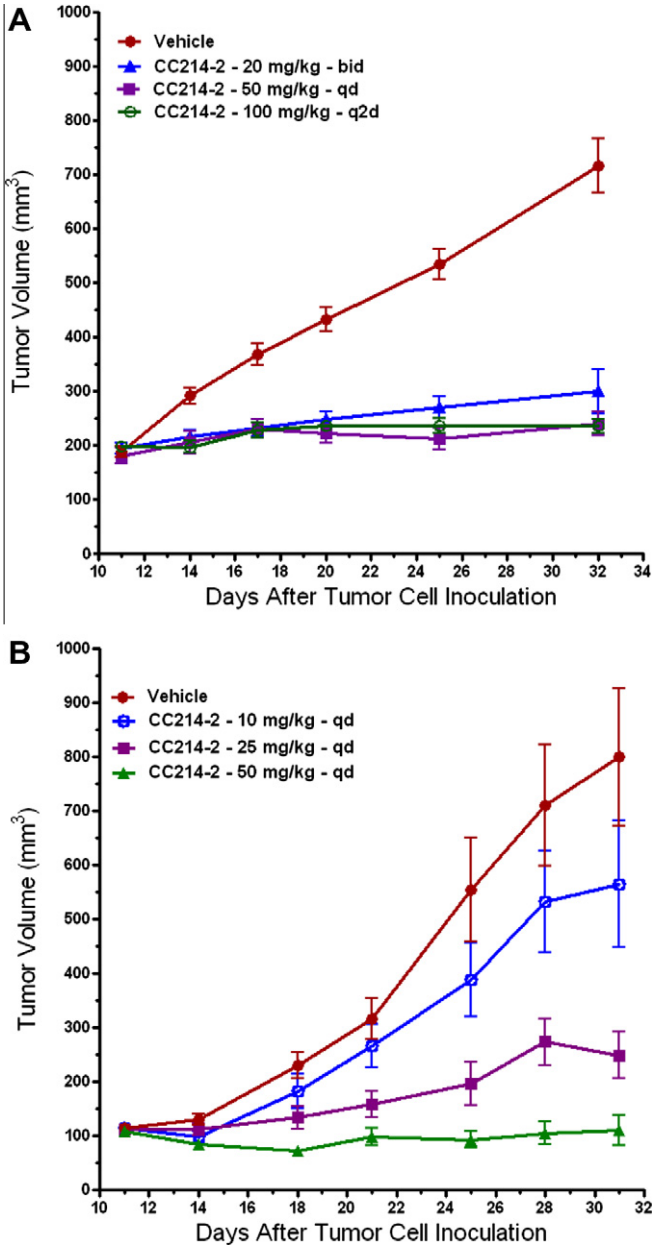


Figure 2. Antitumor activity of CC214-2 in PC3 prostate cancer xenograft model. Activity was determined at various dosing schedules (A) and dose levels with once daily dosing (B).

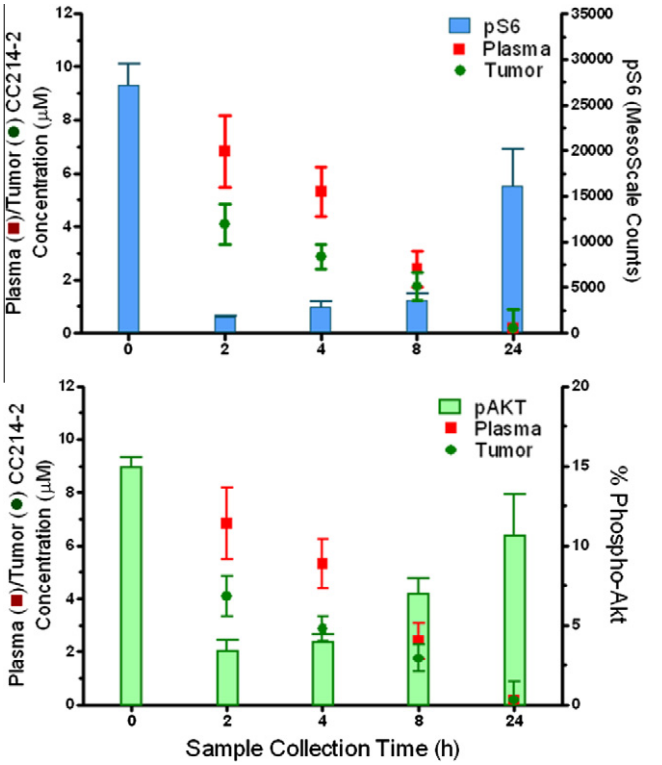


Figure 3. PK/PD relationship of CC214-2 in mice with PC3 tumors with a single oral dose of 30 mg/kg. Inhibition of pS6 (A) and pAktS473 (B) in the tumors was correlated with the compound levels in both plasma and tumors.

of cellular proliferation as a functional effect of mTOR pathway inhibition in these cells.

While analogs such as **9a** and **10a** demonstrated low nanomolar on-target potency and corresponding cellular efficacy, these generally suffered from poor oral bioavailability.[7] Compound **9a**, CC214-1, proved to be useful as an in vitro tool compound for the exploration of mTOR kinase biology.

Comparison of matched-pair **9b** and **10b** shows the ring-expanded core **10b** provides improved cellular efficacy, as assessed by both biomarker and proliferation assays, relative to the parent imidazo[4,5-*b*]pyrazin-2-one **9b**. Compound **10b**, also showed excellent oral bioavailability in rodent, allowing for the exploration of mTOR kinase in in vivo sytems.[7]

Compound **10b**, CC214-2, was further profiled for overall kinase selectivity. When tested in a single point assay against 249 kinases,[8] only one kinase other than mTOR was inhibited >80% at 10 μM. Generation of concentration-response curves for the one kinase, FMS, yielded an IC_{50} value of 2.70 μM.

The antitumor activity of CC214-2 in PC3 xenograft model was initially determined using a number of oral dosing paradigms; once or twice daily or every second day.[9] CC214-2 significantly inhibited PC3 tumor growth under these dosing regimens (Fig. 2A). The activity was further explored using a number of dose levels with a once daily dosing regimen.[10] In this study, CC214-2 significantly inhibited tumor growth in a dose dependent manner, demonstrating 18%, 63% and 84% tumor volume inhibition compared to vehicle control at 10, 25 and 50 mg/kg, respectively (Fig. 2B). The compound was well tolerated at all dose levels and schedules following 3 weeks of treatment.

In order to determine the extent of mTOR pathway inhibition and PK/PD relationship, PC3 tumor-bearing mice were administered with a single dose of CC214-2, and plasma and tumor samples were collected at various time points for analysis. Significant inhibition of mTOR pathway markers pS6 and pAktS473 was observed, indicating that the antitumor activity was mediated through the inhibition of both mTORC1 (pS6) and mTORC2 (pAktS473). When dosed at 100 mg/kg, CC214-2 achieved full biomarker inhibition in the PC3 tumor model through 24 h and compound levels in both tumor and plasma were more than 10-fold above the cellular biomarker IC_{50} values at 24 h.[11] Given the CC214-2 mouse plasma protein binding of 65%, the free plasma concentration at 24 h was 4.6- to 5.7-fold above the pS6 and pAktS473 cellular IC_{50} values. When CC214-2 was dosed at 30 mg/kg, pS6 inhibition was maintained at 87–93% from 2 to 8 h, with 43% inhibition at 24 h (Fig. 3A). Similarly, inhibition of pAktS473 from 2 to 8 h was 53–77% and fell to 28% at 24 h (Fig. 3B). The level of biomarker inhibition correlated well with the plasma and tumor level of compound, which was almost completely cleared by 24 h (Fig. 3). In the 30 mg/kg study, significant biomarker inhibition was observed in tumors at time points

where free plasma concentrations were maintained 7.5- to 2-fold above the cellular biomarker IC_{50} values (2–8 h).

In summary, through use of a ring-expansion core modification to our initial compound series, we have discovered a novel series of 4,6-disubstituted-3,4-dihydropyrazino[2,3-*b*]pyrazin-2(1*H*)-one mTOR kinase inhibitors, with exquisite kinase selectivity. This work led to the identification of CC214-2, a selective and potent inhibitor of mTOR kinase. CC214-2 significantly inhibited PC3 tumor growth in a dose and schedule-dependent manner and has further shown, in vitro and in vivo, potent inhibition of both mTORC1 (pS6) and mTORC2 (pAktS473).

Acknowledgments

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Supplementary data

Supplementary data (biological data with standard deviation and rat PK for CC214-2 (**10b**) and kinase selectivity panel for CC214-1 (**9a**) and CC214-2 (**10b**), synthetic experimental procedures and assay protocols associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.110. These data include MOL files and InChiKeys of the most important compounds described in this article.

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7. Compound **9a**: rat oral F% 0.85. Compound **10a**: rat oral F% 0 (BLQ). Compound **10b**: rat oral F% 100, rat iv CL 8.1 mL/min/kg.
8. SelectScreen® Kinase Profiling Services: Life Technologies, Inc., Carlsbad CA.
9. CB17 SCID mice were inoculated subcutaneously with 2 × 106 PC3 prostate cancer cells. On day 11 when the tumors were established and reached approximately 175 mm^3, the mice were randomized and treated either once or twice daily or every second day orally with vehicle or CC214-2 at a dose volume of 5 mL/kg. CC214-2 was formulated as a suspension in 0.5% CMC/0.25% Tween 80 in water.
10. CB17 SCID mice were inoculated subcutaneously with 2 × 106 PC3 prostate cancer cells. On day 11 when the tumors were established and reached approximately 125 mm^3, the mice were randomized and treated once daily orally with vehicle or CC214-2 at a dose volume of 5 mL/kg. CC214-2 was formulated as a suspension in 0.5% CMC/0.25% Tween 80 in water.
11. CB17 SCID mice with PC3 tumors were administered with a single dose of CC214-2 at 30 or 100 mg/kg orally. CC214-2 was formulated as a suspension in 0.5% CMC/0.25% Tween 80 in water. Plasma and tumor samples were collected at 2, 4, 8 and 24 h and processed for compound exposure analysis. Tumors were also processed for biomarker inhibition using MSD pS6RP(S234/236) and pAktS473.