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Potent and selective thiophene urea-templated inhibitors of S6K

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ABSTRACT

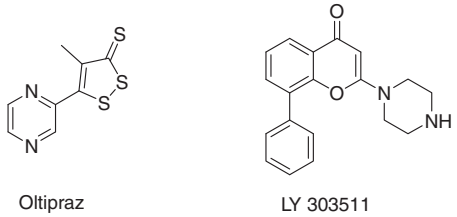
S6K1 (p70 S6 kinase-1) is thought to play a critical role in the development of obesity and insulin resistance, thus making it an attractive target in developing medicines for the treatment of these disorders. We describe a novel thiophene urea class of S6K inhibitors. The lead matter for the development of these inhibitors came from mining the literature for reports of weak off-target S6K activity. These optimized inhibitors exhibit good potency and excellent selectivity for S6K over a panel of 43 kinases.

Activation of the mammalian target of rapamycin (mTOR) S6K1 signaling by nutrients has received broad attention due to its implication in obesity.[1] Morbidly obese patients are often affected by increased insulin resistance, resulting in the development of type II diabetes.[2] Insulin resistance can be regulated by mTOR complex1 activation of p70 S6 kinase-1 (S6K1).[3,4] Recent studies have shown that S6K1-deficient mice exhibit increased lipolysis and reduced adipose tissue mass. They also demonstrate that S6K1-deficient mice are protected from diet- and age-induced obesity making S6K1 an attractive target.[4]

S6K1 is a serine/threonine kinase that phosphorylates the ribosomal protein, S6. Insulin and nutrients activate S6K1 to regulate protein synthesis. However, prolonged activation of S6K1 also leads to the development of insulin resistance. Recent studies revealed that insulin receptor substrate 1 (IRS1) S302, which is proximal to the IRS1 phosphotyrosine-binding (PTB) domain and contains an S6K1-recognition motif, is phosphorylated by S6K1.[5,6] Phosphorylation of S302 disrupts the ability of the PTB domain to interact with activated insulin receptor (IR), leading to decreased insulin signaling.[5] This suggests that S6K1 may have a critical function along with other signaling components in development of obesity and insulin resistance, and may be an important drug target in the treatment of patients suffering from these pathological disorders.

Despite increased reports suggesting S6K1 as a potential pharmacological target, there are only limited classes of small molecule inhibitors reported to date, such as Oltipraz and dithiolethione derivatives,[7–9] and LY303511 (Fig. 1).[10] Our interest in pursuing non classical binding inhibitors led us to the discovery of a series of thiophene ureas as potent and selective S6K1 inhibitors (Fig. 2).

Our studies began with the synthesis of compound **1** (Table 1), which was previously reported as a p38 inhibitor with an off-target effect on S6K1.[11] However, compound **1** showed no enzymatic activity in an ADP hunter assay, a fluorescent intensity assay monitoring the release of ADP product after phosphorylation of substrate. When the nitrogen on heteroaryl-aryl ether was moved from the 4 to the 3 position, compound **2**, the IC_{50} dropped into the measurable range at 9 μM. Activity was lost in conformationally constrained analog **3**. Replacement of the terminal pyridyl group



Oltipraz LY 303511

Figure 1. S6K1 inhibitors.

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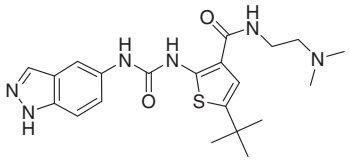
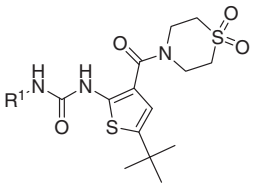
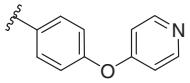
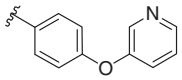
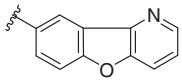
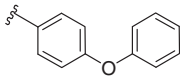
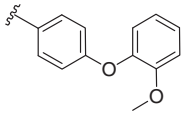
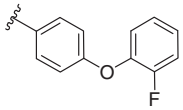
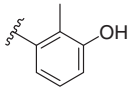
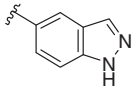


Figure 2. Selective S6K1 inhibitor.

Table 1
S6K1 enzymatic activity of R^1-modified thiophene ureas

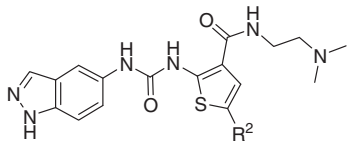


Compound	R^1	S6K1 IC_{50} (μM)
1		>20
2		**9**
3		>20
4		>20
5		>20
6		>20
7		**4**
8		**0.034**

with aryl and substituted aryl also resulted in a loss of activity (**4**–**6**). These preliminary results suggested that a hydrogen bond acceptor with a certain conformation is critical to the activity. Clipping off the terminal ring displayed a modest gain in potency to 1.4 μM (**7**). Replacement of the phenol with a 5-amino indazole resulted in highly active compound **8** (0.034 μM). The indazole moiety possibly interacts with the hinge region of the S6K1 binding pocket. There are a number of publications which depict indazole as a pharmacophore for kinase inhibitors.[12,13]

Thiophene 5-position substitution is critical to the activity. Table 2 details these R^2 substituents. The large *t*Bu proved to be the optimal substituent tested (**9**), followed closely by isopropyl (**10**) then ethyl, cyclopropyl and methyl (**11**–**13**) in a clear size-dependant trend (15, 29, 62, and 233 nM respectively). A phenyl substitution in the R^2 position (**14**) was not tolerated, dropping the potency to 1.9 μM. Both enantiomers of 1-ethanol substituted compounds **15** and **16** are significantly less potent than the isopropyl compound (**10**), suggesting that R^2 substituents fit in a hydrophobic pocket of S6K.

Table 2
S6K1 enzymatic activity of R^2-modified thiophene ureas



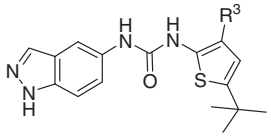
Compound	R^2	S6K1 IC_{50} (nM)
9		15
10		29
11		62
12		70
13		223
14		1900
15		196
16		379

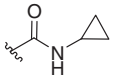
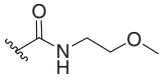
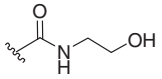
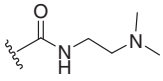
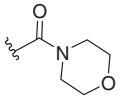
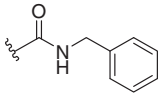
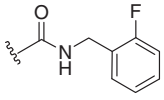
Complete removal of the amide group at the thiophene 3-position from compound **8** maintains activity as illustrated by compound **17** (Table 3). This suggests that the R^3 position lacks key interactions with S6K binding pocket, and instead leads to solvent or an open part of the binding pocket. Taking advantage of possible solvent exposure of R^3, we therefore explored the tolerance of functional groups that can be used to modulate physiochemical properties of the series. Table 3 shows the modifications and their respective solubility and permeability data. The carboxylic acid (**18**) and primary aliphatic amines (**19**–**21**) retain potencies in the 100 nanomolar range. Compounds **9**, **18** and **21** have increased solubility, while compound **18** shows good permeability. The tolerance of R^3 substitution has steric limits. Secondary cyclic amine (**22**) and benzyl amines (**23**–**24**) led to a decrease in potency to 200 nanomolar range. Taken together, a possible binding model incorporating SAR of the R^1, R^2, and R^3 positions is illustrated in Figure 3. Interactions of compound **9** with the hinge region, a hydrophobic pocket and a solvated region are depicted. While the central urea moiety has been shown to bind to p38 in a DFG out configuration,[14] the configuration for binding of this series to S6K can only be fully revealed with an X-ray co-crystal structure.

Several of the compounds were tested in a whole cell activity assay measuring the phosphorylation of ribosomal protein S6. Often there can be a disconnect between the activity of a compound in an enzymatic assay compared to a cellular assay. We found this to be the case for this series of S6K inhibitors. For example, the cellular IC_{50} of **19** was 0.685 μM, about 70-fold less active than enzymatic activity. Similarly, the cellular IC_{50} of **9** was 0.521 μM, about 40-fold less active. This discrepancy between assay results can be due to the differences between the interactions of the compound with a purified recombinant enzyme compared to its interactions with the endogenous enzyme; differences in ATP concentrations in the assays can also contribute. In addition to the inherent differences between the enzymatic and cellular assays, poor cell permeability could also account for the difference in measured inhibitory activity.

The synthesis of the thiophene urea analogs such as **9** is depicted in Scheme 1. 2-Amino thiophene (**27**) was synthesized

Table 3
R^3-modified thiophene ureas



Compound	R^3	S6K1 IC_{50}[a] (nM)	Solubility[b] (μg/mL)	PAMPA[c] (e-6 cm/s)
17	H	19	7	3
18	COOH	52	143	17
19		10	10	4
20		7	10	4
21		19	41	5
9		15	171	2
22		207	n/a	n/a
23		201	n/a	n/a
24		191	n/a	n/a

[a] Enzymatic activity.
[b] Kinetic solubility was measures at pH 6.5 in 50 mM phosphate buffer.
[c] Permeability was measured across a synthetic lipid bilayer.

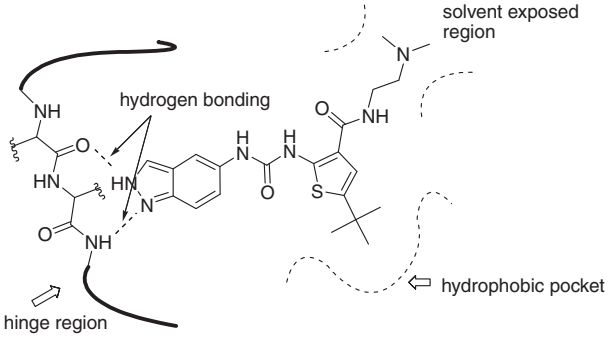
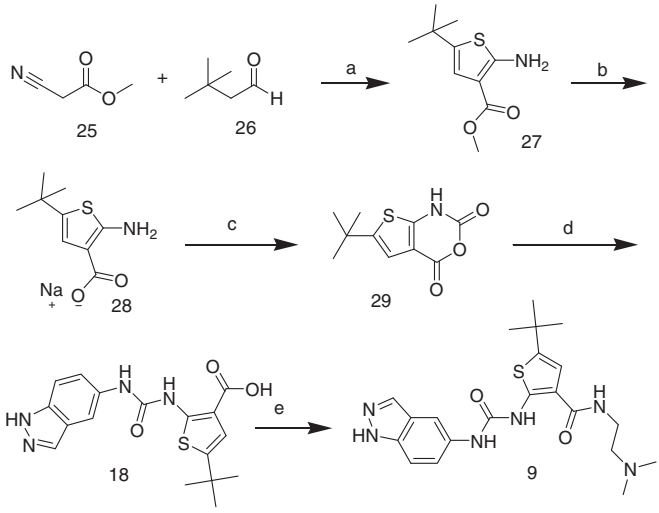


Figure 3. Possible pharmacophore model.

through the Gewald reaction.[15,16] Saponification of **27** gave the corresponding carboxylate acid salt (**28**). Thiaisatoic anhydride (**29**) was prepared as reported.[17] Ring opening of **29** by 5-aminoindazole gave the carboxylic analog **18**. The de-carboxylation product **17** (Table 3) was isolated when reaction temperature increased to 150 °C under microwave conditions. Carboxylic product **18** coupled with the amine to give final product **9**. Compound **9** was isolated by column chromatography. All compounds gave satisfactory spectral and analytical data.[18]

Several compounds were selected for in vitro pharmacokinetics and toxicity evaluation, the results of which are reported in Table 4.



Scheme 1. Reagents and conditions: (a) sulfur, TEA, DMF, 50 °C to rt overnight (68%); (b) $NaOH/H_2O$, microwave, 150 °C, 30 min; (c) triphosgene, toluene, rt, 2 h (83%); (d) 5-aminoindazole, TEA, ACN, 50 °C, 4 h or microwave, 100 °C, 30 min; (e) *N*,*N*-dimethylethane-1,2-diamine, HATU, DIPEA, DMF, rt 1 h (81%).

Table 4
In vitro metabolic stability, CYPs, and dofetilide interaction

Compounds	**9**	**17**	**18**	**19**	**20**	**21**
HLM stability_$T_{1/2}$[a] (min)	38	55	>120	>120	>120	>120
HHEP stability_$T_{1/2}$[b] (min)	125	n/a	n/a	138	367	263
CYP1A2[c] (% inh)	18	36	6	40	45	52
CYP2C9 (% inh)	0	52	55	11	14	30
CYP2D6 (% inh)	7	20	0	12	0	9
CYP3A4 (% inh)	0	4	0	11	2	0
Dofetilide[d] (% inh)	28	0	0	0	0	0

[a] Compound stability in human liver microsomes at 1 μM. LC/MS/MS detection.
[b] Compound stability in human hepatocyte at 1 μM. LC/MS/MS detection.
[c] Compound drug–drug interaction at 3 μM using clinical substrate probes in HLM. % Inhibition reported for CYP3A4, 2D6, 2C9, 1A2. LC/MS/MS detection.
[d] Measured by the displacement of a known hERG channel inhibitor, dofetilide. % Inhibition reported.

Human liver microsomal (HLM) lability assays are useful for determining the clearance of a compound due to Phase I (including cytochrome P450) metabolism. The series shows moderate to good stability in HLM. Hepatocytes are an ideal in vitro system to monitor hepatic metabolism since these intact cells contain all the hepatic enzymes found in in vivo. Human hepatocyte (HHEP) stability assay of the series suggests that they have low metabolic liability. Percent inhibitions for CYP3A4, 2D6, 2C9, and 1A2 are reported in Table 4 as an early indication of possible drug–drug interactions. The series shows no or low risk of inhibition against 3A4 and 2D6. Compounds **17**, **19**, **20**, and **21** showed moderate risk against 1A2, while **17**, **18**, and **21** showed moderate risk against 2C9. Assessment for QT interval elongation measured by displacement of a known hERG potassium ion channel inhibitor, dofetilide, showed no risk.[19]

Selectivity was examined against a panel of 43 kinases (Fig. 4). The series was highly selective for inhibition of S6K1. For example, the heat map of percent inhibition of compound **18** against other kinases was cold and clean, only JAK3, CLK1, and CHEK2 showed low levels of inhibition.

In summary, a novel series of small molecule S6K1 inhibitors with good potency, good physiochemical properties, and excellent selectivity profile was reported.

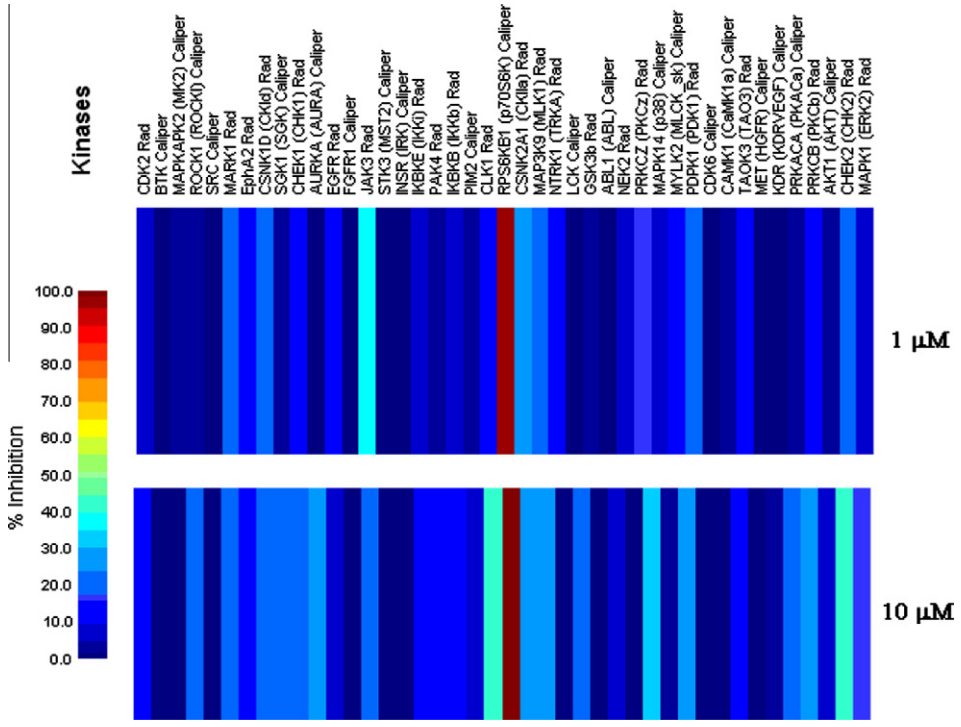


Figure 4. Selectivity profile of compound **18** against 43 kinases.

Acknowledgments

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18. Selected experimental and analytical information: all ^{1}H NMR data were recorded by a 400 MHz Bruker NMR spectrometer. DMSO-d_6 was used as NMR solvent. All compounds >95% purity by HPLC (UV, 254 nm) and ^{1}H NMR.
Compound **27**: methyl 2-amino-5-*tert*-butylthiophene-3-carboxylate: triethylamine (225 mg, 2.5 mmol) was added to a vigorously stirred mixture of cyanoacetic acid methyl ester (500 mg, 5 mmol), sulfur powder (162 mg, 5 mmol) and DMF (10 mL) at 50 °C. 3,3-Dimethylbutyraldehyde (505 mg, 5 mmol) in 4 mL of DMF was added dropwise to this suspension, while maintaining the temperature at 50 °C. When the addition was complete, the reaction mixture was allowed to cool to rt and stirred overnight. The reaction mixture was partitioned in water and ether. The ether layer was washed with water (15 mL × 4), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford a yellow solid, 68% yield. *m*/*z* ES+ = 214.08, calcd 214.08; ^{1}H NMR δ ppm 7.15 (s, 2H), 6.48 (s, 1H), 3.69 (s, 3H), 1.25 (s, 9H).
Compound **29**: 6-*tert*-butyl-1*H*-thieno[2,3-*d*][1,3]oxazine-2,4-dione: compound **27** (63 mg, 0.30 mmol), 2-aminothiophene and 0.6 mL of 1 M NaOH aqueous solution were combined in a conical bottom microwave vial. The slurry was irradiated under microwave at 150 °C for 30 min. Triphosgene (44 mg, 0.15 mmol) in 1 mL toluene was added slowly with vigorous stirring. The resulting mixture was allowed to stir at rt for 2 h. The precipitate was filtered and washed successively with water, hexane and ether, then dried in vacuo to give compound **29** with 83% yield. *m*/*z* ES+ = 226.09, calcd 226.05; ^{1}H NMR δ ppm 12.60 (s, 1H), 6.92 (s, 1H), 1.23 (s, 9H).
Compound **18**: 2-(3-(1*H*-indazol-5-yl)ureido)-5-*tert*-butylthiophene-3-carboxylic acid: compound **29** (125 mg, 0.56 mmol), 5-aminoindazole (74 mg, 0.56 mmol) and 4.5 mL of acetonitrile were added to a 5 mL microwave vial After the subsequent addition of neat triethylamine (154 μL, 1.11 mmol), the resulting mixture was irradiated under microwave at 100 °C for 30 min. The solvent was removed in vacuo. Flash column chromatography (0–10% MeOH in dichloromathane, linear gradient over 30 min) provided compound **18** as a off-white solid. *m*/*z* ES+ = 359.32, calcd 359.11; ^{1}H NMR δ ppm 12.94 (br s, 1H), 10.05 (s, 1H), 8.00 (s, 1H), 7.99 (d, *J* = 1.20 Hz, 1H), 7.48 (d, *J* = 8.84 Hz, 1H), 7.36 (dd, *J* = 8.84, 1.60 Hz, 1H), 6.77 (s, 1H), 1.32 (s, 9H). Urea NH absent.
Compound **9**: 1-(5-*tert*-butyl-3-((2-(dimethylamino)ethyl)carbamoyl)thiophen-2-yl)-3-(1*H*-indazol-5-yl)urea: compound **18** (1.79 g, 5 mmol), *N*,*N*-dimethylethane-1,2-diamine (0.44 g, 5 mmol), and 40 mL of DMF were added to a round bottom flask and stirred until completely dissolved. Diisopropylethylamine (776 mg, 6 mmol) was added, followed by HATU (2.09 g, 5.5 mmol). The resulting solution was stirred at rt for 1 h. The solvent was removed in vacuo. Flash column chromatography (NH_4OH/MeOH/DCM, 0.5:5:94.5) gave compound **9** with 81% yield. *m*/*z* ES+ = 429.29, calcd 429.20; ^{1}H NMR δ ppm 12.95 (s, 1H), 11.35 (s, 1H), 10.06 (s, 1H), 8.14 (t, *J* = 5.84 Hz, 1H), 7.99 (s, 2H), 7.47 (d, *J* = 8.84 Hz, 1H), 7.36 (dd, *J* = 8.84, 1.64 Hz, 1H), 7.11 (s, 1H), 3.28–3.41 (m, 2H), 2.40 (t, *J* = 6.95 Hz, 2H), 2.19 (s, 6H), 1.34 (s, 9H).
19. Greengrass, P. M.; Stewart, M.; Wood, C. M. Patent WO2003021271, 2003.