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Conformationally-restricted cyclic sulfones as potent and selective mTOR kinase inhibitors

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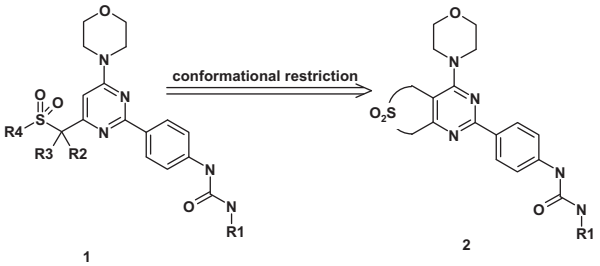
ABSTRACT

Novel conformationally-restricted mTOR kinase inhibitors with cyclic sulfone scaffold were designed. Synthesis and structure–activity relationship (SAR) studies are described with emphasis on optimization of the mTOR potency and selectivity against class I PI3Kα kinase. PF-05139962 was identified with excellent mTOR biochemical inhibition, cellular potency, kinase selectivity and in vitro ADME properties.

Mammalian target of rapamycin (mTOR) signaling pathway plays a central role in driving tumor cell proliferation, survival, angiogenesis and metastasis by responding to nutrients, growth factors, and cellular energy level.[1] mTOR is a mammalian serine/threonine kinase discovered in 1994 and a member of PI3K like kinase (PIKK) family of proteins. mTOR exists in two different complexes,[2] mTORC1, a rapamycin sensitive complex signaling to S6K1 and 4E-BP1 to promote translation and cell growth; and mTORC2, an rapamycin insensitive complex signals to AKT, an important oncoprotein that activates a broad anti-apoptotic mechanism for cell survival. These two distinct mTOR components function through two different sets cell regulations. Rapamycin analogs (rapalogues) are protein–protein inhibitors and allosteric inhibitors of mTOR through mTORC1 but not mTORC2.[3]

Rapalogues such as RAD001 (everolimus, Novartis), CCI-779 (temsirolimus, Pfizer), and AP23573 (ridaforolimus, deforolimus, Merck) entered anti-tumor clinical trials recently.[4] Despite the high expectation for their application in oncology based on sound rationale related to the presumed mechanism-of-action, the rapalogues have only met with modest success. Most notable is the utility of these agents as monotherapy in renal cell cancer (RCC) and mantle cell lymphoma. Existence of this rapamycin insensitive component of the mTOR signaling pathway thus provides new opportunities to further inhibit mTOR related signaling. A mTOR kinase inhibitor should prevent signaling through both mTORC1 and mTORC2 to have a broad and advantageous spectrum of pharmacology over rapalogues to exploit the full therapeutic potential of targeting mTOR. In addition, there are reports that rapalogues led to PI3K/AKT activation and attenuated the agents' antitumor activities.[5] It remains to be seen if the same activation is observed in selective mTOR kinase inhibitors.

To find high quality leads of mTOR kinase inhibitors, we started our own HTS and literature search in parallel. We were drawn to a series of low molecular weight mTOR inhibitors published by Astrazeneca colleagues[6] as general structure **1** (Scheme 1). The simple morpholinylpyrimidine core in these compounds provides us a great opportunity to find other novel templates by scaffold-hopping. After analyzing literature data on this series of compounds, we found that most of these analogues have either small alkyl groups or cyclic propyl group (as R2 and R3 in general struc-



1 2

Scheme 1. Design of conformationally-restricted cyclic sulfones **2**.

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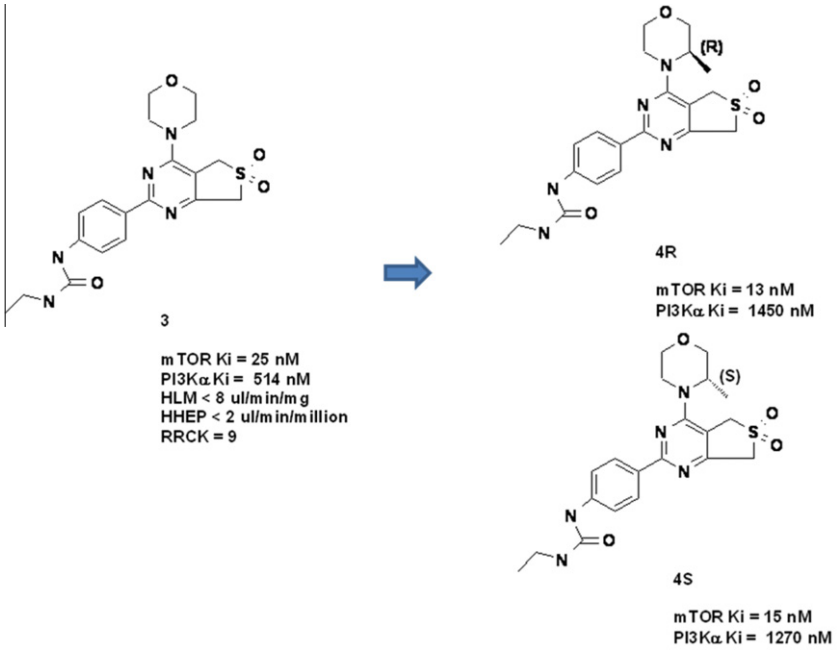
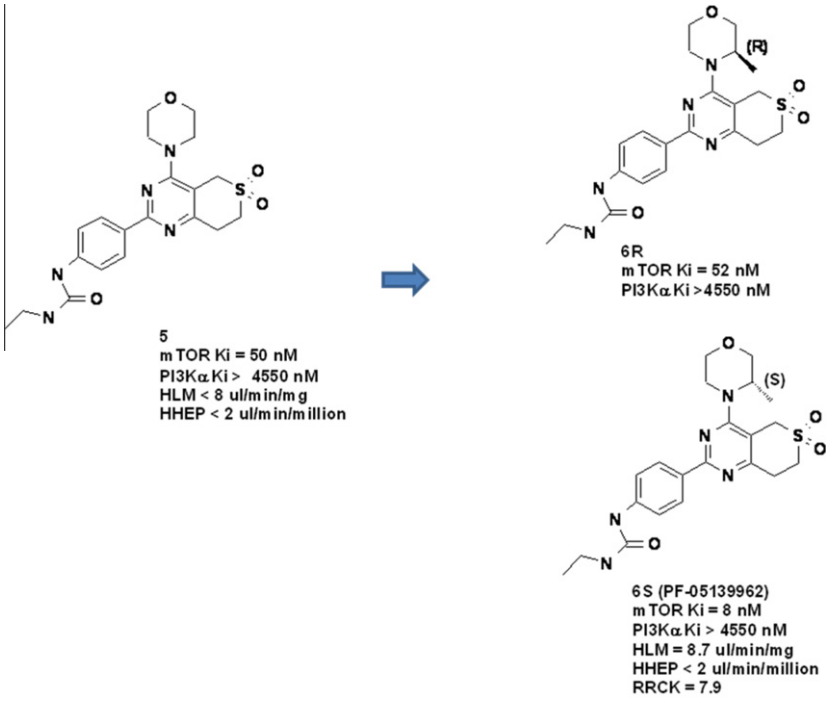


Scheme 2. Both the (*R*) and (*S*) methyl morpholines have about the same potency and selectivity.



Scheme 3. PF-05139962, a potent and selective mTOR inhibitor.

ture **1**) on the carbon in between pyrimidine core and the sulfonyl side chain. Possible reasons for this chemical modification are (1) to increase metabolic and chemical stability of the sulfonyl side chain, (2) to restrict movement of the sulfonyl side chain, (3) to stabilize a favorable binding conformation for better potency. Based on these assumptions, we decided to make a series of conformationally-restricted cyclic sulfones (**2**, in Scheme 1) to test our hypothesis.

5-Membered ring cyclic sulfone (**3**) with plain morpholine was made first for testing, and encouraging result was obtained as shown in Scheme 2. Compound **3** has mTOR K_i = 25 nM and PI3Kα K_i = 514 nM. It has about 20-fold selectivity over PI3Kα. The compound has low in vitro clearance as indicated by the low human liver microsomes (HLM) and human hepatocyte (HHEP) clearance. In addition, the compound should have good permeability based on its good RRCK[7] (Scheme 2). It is important for kinase inhibitors to have good cell permeability to be biochemical efficient and show good cellular potency.

Methyl group was introduced to the morpholine moiety to see if we can get better potency of mTOR and selectivity against PI3Kα by

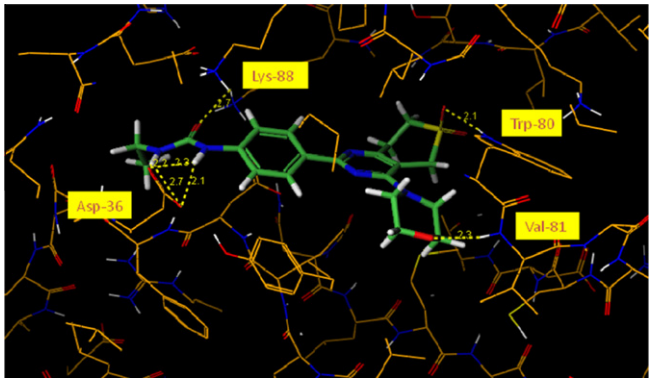


Figure 1. PF-05139962 purposed binding in mTOR.

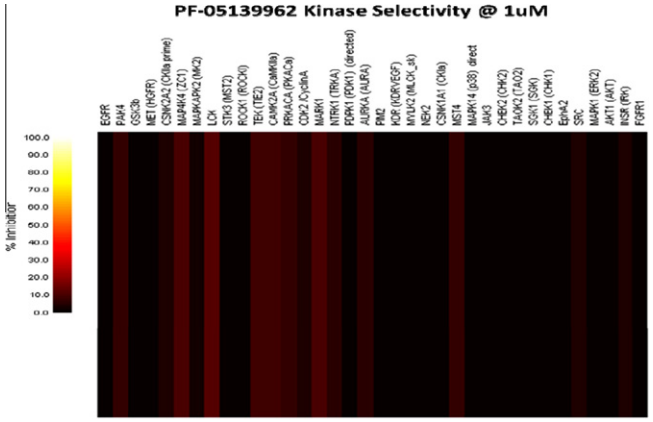
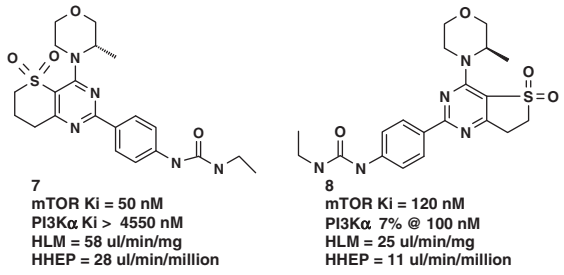


Figure 2. PF-05139962 kinase selectivity at 1 μM.

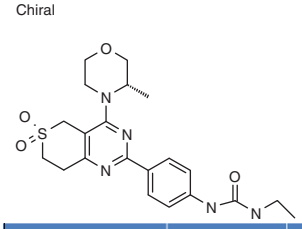


Scheme 4. Different region-isomers.

increasing steric bulkiness and further restricting conformation of the molecule. We did see selectivity against PI3Kα improvement from 20× to 80–100× between **3** and **4R** or **3** and **4S**. However, the mTOR biochemical activity of **4R** or **4S** was only slight improved over compound **3**. Interestingly, compounds **4R** and **4S** are about equal potent for both mTOR and PI3Kα regardless the different stereochemistry of the methyl group (Scheme 2). With these interesting data, we decided to expand the SAR to 6-membered ring cyclic sulfone and walk the sulfone group to different positions in the ring.

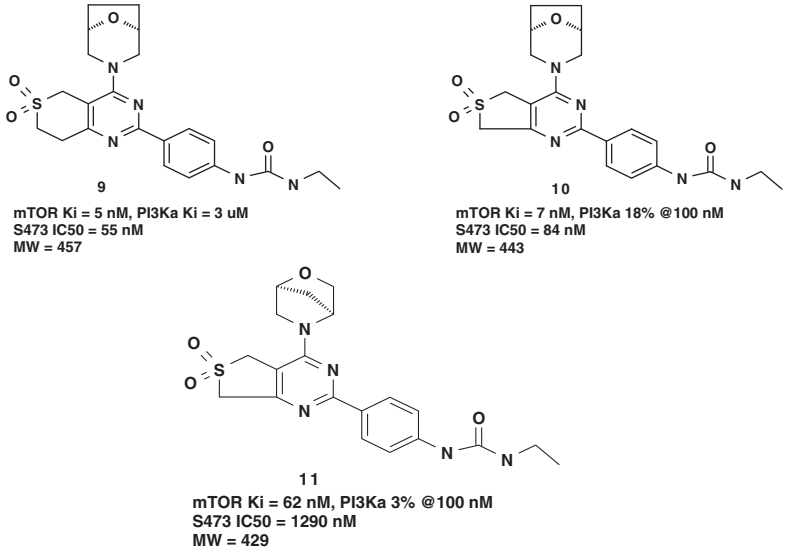
Compound **5**, the 6-membered ring cyclic sulfone as the homo-analog of compound **3**, is 2× less potent than compound **3** on mTOR kinases but it has PI3Kα K_i more than 4.5 μM. After introducing methyl group to the morpholine moiety, both compounds **6S** (PF-05139962) and **6R** are also inactive to PI3Kα K_i more than 4.5 μM. The 6-membered ring cyclic sulfone appears to be a better template for selectivity because of lacking PI3Kα activity. We are pleased to see that these compounds still keep good ADME profile (Scheme 3). Most interestingly and contrary from what was ob-

Table 1
PF-05139962 rat PK, no in vitro in vivo correlation

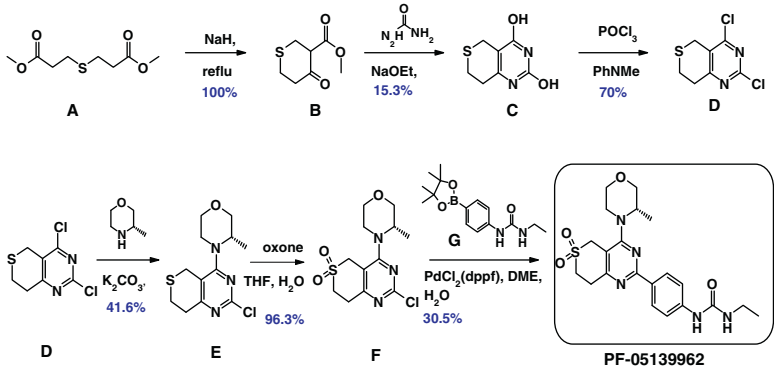


CL	mL/min/kg	66.2
Vdss	mL/kg	2.56
T1/2	Hours	1.05
F	%	30
fe	%	5.5

	Predicted CL with PB	Predicted CL without PB	Observed CL	Obs/Pred With PB
Compound	mL/min/kg	mL/min/kg	mL/min/kg	
PF-05139962	5.69	33	66.2	11.63



Scheme 5. Other morpholine replacements.



Scheme 6. Synthesis of PF-05139962.

served in the corresponding 5-membered ring sulfones (**4R** and **4S**), compound **6S** with (*S*)-methyl-morpholine moiety has mTOR K_i = 8 nM which is superior to its stereoisomer **6R** (mTOR K_i = 52 nM).

Compound **5** was docked to our mTOR homogenous model as shown in Figure 1. According to this model, the morpholinyl oxygen interacts with Val-81 in the hinge region, oxygen of the sulfone interacts with Trp-80 and the urea side chain play a critical role in interacting with Lys-88 and Asp-36. Several attempts to replace the urea group with other bioisosteres failed to deliver compounds with decent mTOR inhibition since the flexible and free urea side chain can maximize the H-bond interactions with Lys-88 and Asp-36.

Two region-isomers, compounds **7** and **8** were synthesized (Scheme 4). However, they are less potent than their corresponding counter partners compounds **6S** and **4R** respectively. Compound **7** also has higher clearance in HLM and HHEP as well.

We then moved our attention to modify the morpholine portion of the molecule since data suggests this portion of molecule is related to compounds potency and selectivity. Among these modified morpholine analogs, compounds **9–11** are highlighted and they have desired potency and selectivity profiles as shown in Scheme 5. Especially, compound **9** and **10** that also have single digit mTOR K_is, great selectivity against PI3Kα and have pS473 cellular IC_{50} <100 nM.

After further evaluating these sulfones, we decided to focus on PF-05139962 which gives us most balanced profile that we are looking for. As mentioned earlier, PF-05139962 is a potent mTOR inhibitor with great selectivity against PI3Kα and great in vitro ADME profile. It has pS473 and pS6 cellular IC_{50} = 48 and 6 nM respectively. It has great selectivity against other receptors and kinases (Fig. 2 for its kinase selectivity). No genotoxicity was observed on this compound and no more than 25% inhibiton was observed for major CYP enzymes (3A4, 1A2, 2C9, 2D6) at 3 μM. This compound has LE = 0.35 and LipE up to 6.8 which is in a very desirable range for a kinase inhibitor.[8]

PF-05139962 was subjected to in vivo PK studies in rats to further understand the PK-PD for this compound. Although, PF-05139962 has good permeability and low in vitro clearance; high clearance, short $t_{1/2}$ and 30% bioavailability (*F*) were observed in rats. The predicted in vivo clearance based on in vitro data is at lease 10× less than what was observed in in vivo in rats after protein binding correction (Table 1). The reason for this in vitro/in vivo disconnection remains unknown and we decided to halt this series compounds for more evaluation because of the uncertainty on human PK and dose predictions.

The synthesis of PF-05139962 is outlined in Scheme 6. Compound **A** in anhydrous THF was treated with NaH at room temperature then the mixture was heated to reflux to give crude compound **B,** a thiopyranone, as a yellow oil which was used directly for next step without further purification. The crude thiopyranone **B** was treated with urea and NaOEt in EtOH from rt. to 70 °C to yield crude bicyclic compound **C** as a white solid. The crude compound **C** was used directly and chlorinated with $POCl_3$ in the presence of *N*,*N*-dimethylaniline at 100 °C to give compound **D** in 70% yield as a yellow solid. 2-Methylmorpholine was coupled with compound **D** in hot DMF under basic conditions (K_2CO_3) to give compound **E** in 42% yield as a yellow solid. The thioether compound **E** was oxidized with oxone in THF–H_2O 1:1 mixed solvent to generate the corresponding sulfone, compound **F**, as a yellow solid in 93% crude yield. The crude compound **F** was coupled with compound **G**, a phenylurea boronic ester, in the presence of Cs_2CO_3 and PdCl2(dppf) in DME–H_2O mixed solvent at 100 °C under microwave conditions to give PF-05139962 in 30.5% yield after purification as a white solid.

In summary, a series of novel cyclic sulfones were designed based on the concept of conformational restriction to generate potent and selective mTOR inhibitors. Among these inhibitors, PF-05139962 has more than 500-fold selectivity against PI3Kα and good in vitro ADME profile. However, no in vitro in vivo PK correlation was observed in rats and this disconnection confounds our human PK predictions. Compounds in this series were halted for further evaluation. Learning from this series of compounds was applied to develop better selective mTOR inhibitors and will be disclosed in due course.

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