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Discovery of 3,6-dihydro-2*H*-pyran as a morpholine replacement in 6-aryl-1*H*-pyrazolo[3,4-*d*]pyrimidines and 2-arylthieno[3,2-*d*]pyrimidines: ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR)

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ABSTRACT

The morpholine hinge-region binding group on a series of pyrazolopyrimidine and thienopyrimidine mammalian target of rapamycin (mTOR) inhibitors was replaced with 3,6-dihydro-2*H*-pyran (DHP), giving compounds of equivalent potency and selectivity versus PI3K. These results establish the DHP group as a hinge-region binding motif for the preparation of highly potent and selective mTOR inhibitors.

The mammalian target of rapamycim (mTOR), a serine/threonine protein kinase, is a member of the phosphoinositide-3-kinase-related kinase (PIKK) family and is a key mediator of signaling through the PI3K–AKT pathway. The deregulation of PI3K–AKT–mTOR signaling is one of the most common genetic alterations in proliferative diseases and as such has garnered intense interest from industry and academia.[1] mTOR exists in at least two functional complexes, mTOR/raptor (mTORC1) and mTOR/rictor (mTORC2), which play critical roles in the transduction of environmental stimuli into cellular processes, including cellular growth and survival, metabolism, and ribosomal synthesis. mTORC1, which is inhibited by rapamycin analogs, phosphorylates S6K and 4E-BP1.[2] mTORC2, unaffected by rapamycin analogs, activates AKT via phosphorylation of serine 473.[3] Rapamycin inhibition of mTORC1 has been shown to result in activation of AKT via a feedback mechanism.[4] An ATP-competitive inhibitor is expected to affect both mTOR complexes and thus lead to decreased AKT activity. Since AKT activation has anti-apoptotic, pro-survival effects, ATP-competitive mTOR inhibitors may have increased antitumor effects compared to rapamycin analogs.

We have identified a series of 4-morpholino-6-aryl-1*H*-pyrazolo-[3,4-*d*]pyrimidines that are highly potent and selective ATP-competitive inhibitors of mTOR enzymatic activity, cellular proliferation, and in vivo tumor growth.[5–8] The morpholine group in these compounds forms the crucial hinge region interaction with mTOR via a hydrogen bond between the morpholine oxygen and Val2240.[5–7] In efforts to further improve the potency, selectivity and/or PK/PD properties of our inhibitors we have investigated derivatives of the morpholine group.[9] We here report the successful use of the 3,6-dihydro-2*H*-pyran (DHP) group as an alternative to morpholine as a hinge region binding motif for the design of potent and selective mTOR inhibitors.

Pyrazolopyrimidines incorporating the DHP group were prepared by the route outlined in Scheme 1.[10] The synthetic procedures described herein were not optimized for yield; however, products of sufficient yield and purity for medicinal chemistry purposes were commonly obtained. Compound **1**, 5-amino-1-(2,2,2-trifluoroethyl)-1*H*-pyrazole-4-carbonitrile, was prepared by cyclization of 2,2,2-trifluoroethyl hydrazine with 2-(ethoxymethylene)malononitrile. Benzoylation of the amino group of **1** gave **2**, which upon treatment with refluxing base and peroxide provided **3**, containing the pyrazolopyrimidine core. Heating a neat suspension of **3** with phosphorus oxychloride in a sealed tube gave chloride **4**. Hydrogenation under one atmosphere of hydrogen in the presence of di-*tert*-butyldicarbonate gave **5**. Subjection of **5** to Stille conditions in the presence of tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane[11] gave coupling product **6**. Following removal of the BOC protecting group with trifluoroacetic acid, work-up with aqueous sodium bicarbonate gave aniline intermediate **7**. Treatment of **7** with triphosgene provided the putative intermediate isocyanate to which the respective amines were added, furnishing final ureas **11**–**13**.

As shown in Table 1, the replacement of morpholine in ureidophenyl-bearing 1*H*-pyrazolo[3,4-*d*]pyrimidines **8**–**10** with DHP led to highly potent[12] and selective[13] mTOR inhibitors **11**–**13**. Fluoroethyl urea **12**, particularly, demonstrated substantially weaker activity against PI3Kα, thereby providing a compound with >2000-fold mTOR selectivity. In addition to their enzyme activity and selectivity, DHP containing compounds also generally had comparable antiproliferative potency in LNCap cells[14] and stability in nude mouse microsomes.[15]

Molecular modeling provided an explanation for the similar activities of the morpholine and DHP containing inhibitors. When

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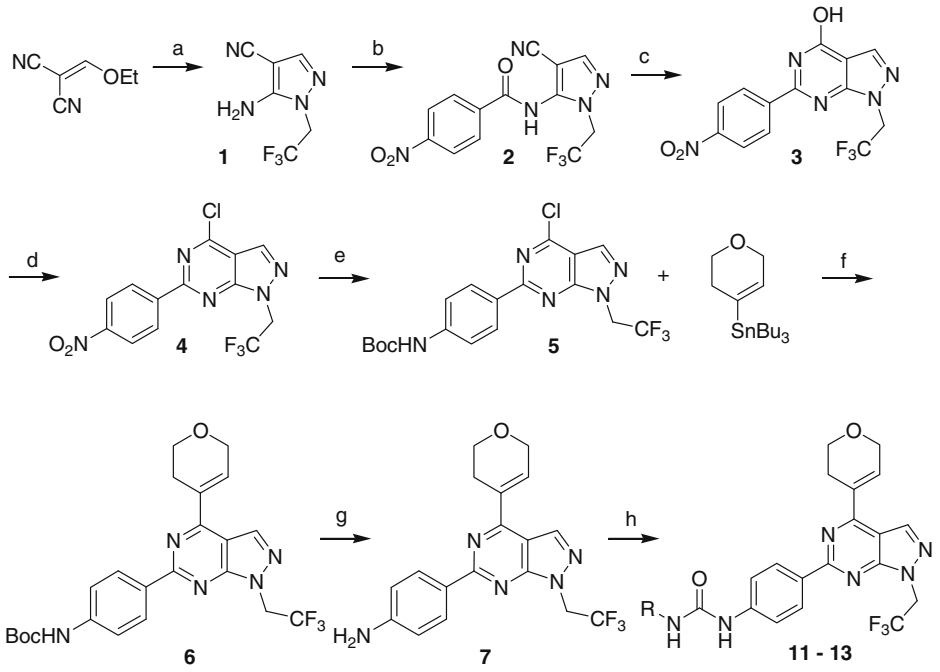
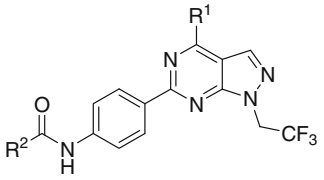
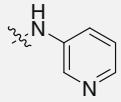


Scheme 1. Reagents and conditions: (a) 70 wt % aq trifluoroethyl hydrazine, ethanol; (b) *p*-NO_2BzCl, CH_2Cl_2, CH_3CN; (c) 30% H_2O_2, 2.5 N aq NaOH, ethanol; (d) $POCl_3$; (e) BOC_2O, H_2, 10% Pd/C, THF; (f) $PdCl_2$ $(PPh_3)_2$, DMF; (g) TFA, CH_2Cl_2, followed by aq $NaHCO_3$ work-up; (h) triphosgene, Et_3N, CH_2Cl_2, then RNH_2.

Table 1
6-Ureidophenyl-l-1*H*-pyrazolo[3,4-*d*]pyrimidines

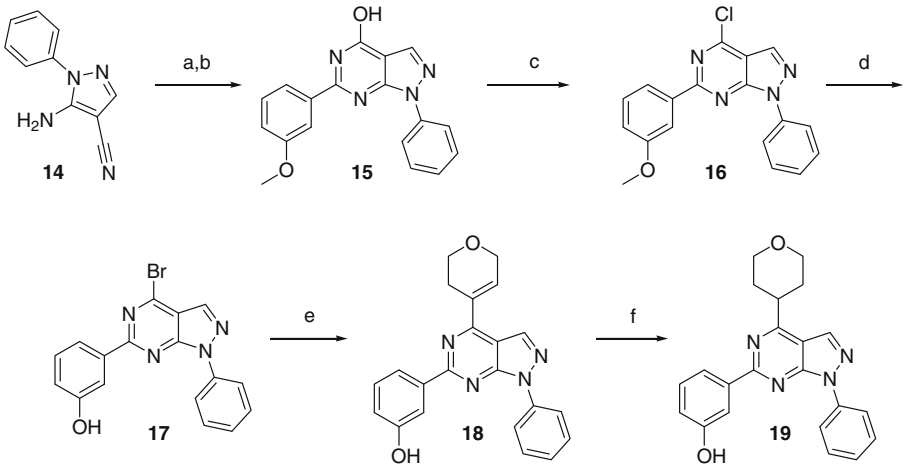


Compd	R^1	R^2	mTOR[a]	P13Kα[a]	Sel.[b]	LNCap[a]	Micros.[c]
11	DHP	$-NHCH_3$	2	200	100	260	13
8	morpholine		1	194	194	230	26
12	DHP	$-NHCH_2CH_2F$	1	2300	2300	72	>30
9	morpholine		0.6	315	525	60	22
13	DHP		1	66	66	380	25
10	morpholine		0.2	10	50	<27	>30

[a] Average IC_{50} (nM). The average error for IC_{50} determinations was <25%.
[b] P13Kα IC_{50}/mTOR IC_{50}.
[c] Nude mouse microsomes $T_{1/2}$ (min).

the inhibitors were docked in an mTOR homology model derived from a PI3Kγ crystal structure,[5] similar binding modes were observed for inhibitors containing morpholine and DHP groups. The oxygen atoms were well overlaid, and similar H-bonds to the hinge region valine were seen for both isosteres. Docking studies also suggested a similar binding mode for compounds in which the double bond in the DHP group had been reduced to give a tetrahydro-2*H*-pyran (THP). Therefore, we explored whether the THP group could also function as a morpholine isostere, by preparation of DHP and THP containing inhibitors on a test system. As outlined in Scheme 2, reaction of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **14** with 3-anisoyl chloride followed by base-catalyzed condensation provided 6-(3-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol **15**. Chlorination using phosphorus oxychloride gave **16** which upon demethylation with boron tribromide gave the bromide **17**. Introduction of the DHP group as a morpholine replacement, by Stille coupling of **17** with tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane, gave **18**. Reduction of the double bond in **18** by hydrogenation gave THP **19**.

Remarkably, reduction of the olefin in **18** to give the THP containing pyrazolopyrimidine **19** led to a profound decrease in mTOR and PI3Kα potency (Table 2). Consideration of the minimum energy conformation of the two inhibitors in solution, however, clarifies the cause of the decreased activity of the THP analog. As shown in Figure 1A, a conformational search of **18** revealed that in the minimum energy conformation the DHP group is co-planar with the pyrazolopyrimidine core. A similar conformation was observed for morpholino-pyrazolopyrimidines (not shown). In con-



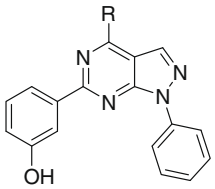
Scheme 2. Reagents and conditions: (a) 3-Anisoyl chloride, Et_3N, CH_2Cl_2; (b) 30% H_2O_2, 2.5 N aq NaOH, ethanol; (c) $POCl_3$; (d) BBr_3, CH_2Cl_2; (e) tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane, $PdCl_2(PPh_3)_2$, DMF; (f) H_2, 10% Pd/C, CH_2Cl_2.

trast, the minimum energy conformation for the THP group is rotated ca. 90° out-of-plane with the core (Fig. 1B). An overlay of the minimum energy conformation of the THP analog (**19**) with the docked binding mode of the DHP compound (**18**) (Fig. 2), highlights a number of clashes of the THP ring with active site residues, specifically Ile2356 and Trp2239. Hence, steric crowding precludes the binding of the THP analog **19** in its minimum energy conformation, forcing the adoption of a co-planar conformation or requiring a conformational shift of the enzyme to accommodate an out-of-plane conformation of the THP group in order to bind mTOR.

We also investigated whether the DHP could function as a morpholine surrogate on other scaffolds. We recently showed that 2-ureidophenyl-4-morpholinothieno[3,2-*d*]pyrimidines also potently inhibited mTOR.[17] DHP containing thienopyrimidines were prepared as outlined in Scheme 3. Thus, treatment of 3-aminothiophene-2-carboxamide **20** with triphosgene gave thienopyrimidine **21**. Chlorination with phosphorus oxychloride gave **22**, which was reacted with tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane under Stille coupling conditions to furnish **23**. Suzuki coupling with the pinacol ester of 4-aminophenylboronic acid was followed by treatment of resulting **24** with triphosgene and amine to give the target urea compounds.

The biological results of the thienopyrimidine series corroborate those of the pyrazolopyrimidine core: the DHP group, as morpholine replacement, gives potent in vitro inhibitors of mTOR (**25**–**29**, Table 3). The mTOR potency of inhibitors derived from this scaffold was slightly lower than that of the corresponding pyrazolopyrimidines (compare **26** to **12**; **28** to **13**) whereas the PI3K activ-

Table 2
Comparison of hinge region binding groups



Compd	R	mTOR[a]	PI3Kα[a]	Sel.[b]
18	DHP	18	59	3.3
19	THP	500	1013	2

[a] Average IC_{50} (nM). The average error for IC_{50} determinations was <25%.
[b] PI3Kα IC50/mTOR IC_{50}.

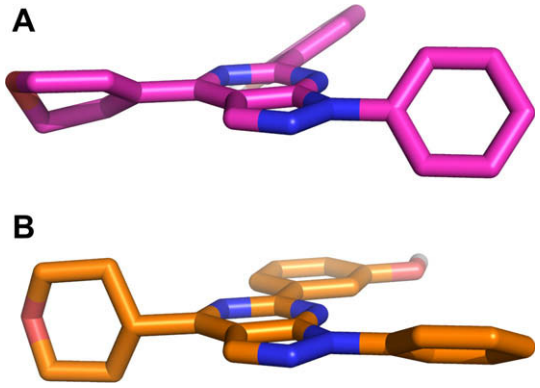


Figure 1. Comparison of minimum energy conformations. (A) DHP **18** (B) THP **19**. ConfGen[16] was used to explore the conformational space of each molecule in water. Redundant conformers were eliminated using an RMSD cutoff of 0.5 Å. The 'thorough' search mode was used and a maximum of 128 ring conformations were generated. The minimum energy conformation from ConfGen for the THP analog was used to overlay with the docked conformation of the DHP analog.

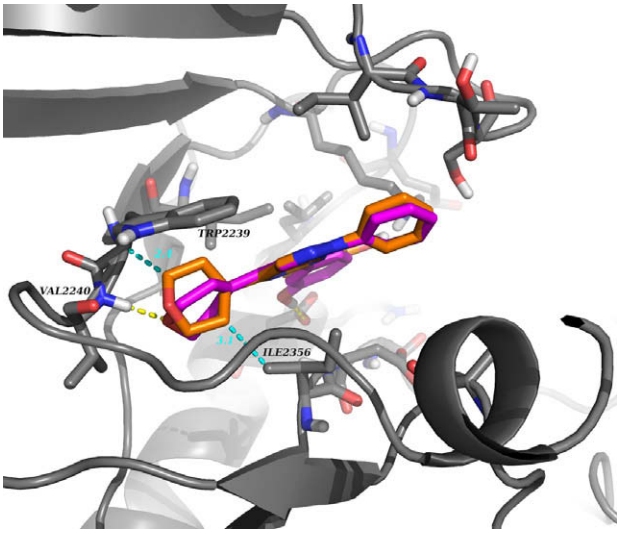
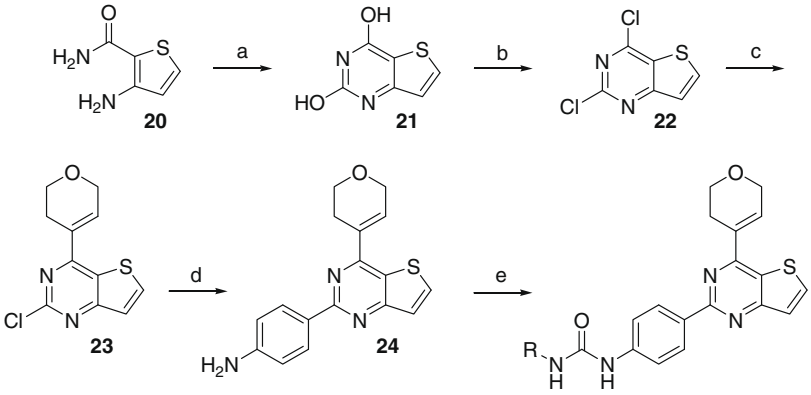
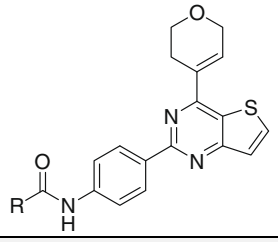


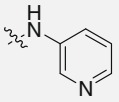
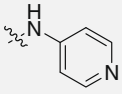
Figure 2. Overlay of docked DHP analog **18** (in magenta) and the minimum energy conformation of THP analog **19** (in orange) in an mTOR homology model based on the PI3Kγ crystal structure. The hydrogen bond between the hinge region and the DHP ring is shown in yellow; steric clashes for the THP analog are shown in cyan.



Scheme 3. Reagents and conditions: (a) Triphosgene; (b) $POCl_3$; (c) tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane, $PdCl_2(PPh_3)_2$, THF; (d) 4-aminophenylboronic acid pinacol ester, $Pd(PPh_3)_4$, $PhCH_3$/EtOH; (e) Et_3N, triphosgene, followed by appropriate amine.

Table 3
2-Ureidophenyl-thieno[3,2-d]pyrimidines



Compd	R	mTOR[a]	P13Kα[a]	Sel.[b]	LNCap[a]	Micros.[c]
25	–$NHCH_2CH_3$	3.3	1053	319	470	3
26	–$NHCH_2CH_2F$	2.5	1373	560	1300	6
27	–NHPh	7.2	28	4	800	21
28		1.1	35	32	210	>30
29		1.8	27	15	200	>30

[a] Average IC_{50} (nM). The average error for IC_{50} determinations was <25%.
[b] P13Kα IC_{50}/mTOR IC_{50}.
[c] Nude mouse microsomes $T_{1/2}$ (min).

ity of the thienopyrimidines was similar to or slightly better than that of the corresponding pyrazolopyrimidines. Consequently, the thienopyrimidines were somewhat less selective for mTOR than the pyrazolopyrimidines and may be of particular value for the development of dual PI3K/mTOR inhibitors. In this respect, pyridinyl ureas **28** and **29** are of interest due to the combination of potent in vitro inhibition and excellent microsomal stability.

In summary, we have identified the 3,6-dihydro-2*H*-pyran (DHP) as a suitable bioisostere for the morpholine group in pyrazolopyrimidine and thienopyrimidine mTOR inhibitors. Molecular modeling suggests that the DHP and morpholine groups form the same essential hydrogen bond to Val2240 in the hinge region of mTOR. The findings in this paper add to our expanding repertoire for the design of mTOR and/or PI3K inhibitors. Through the judicious choice of the urea substituent, scaffold and morpholine replacement, the potency and selectivity of the inhibitors may be modulated in order to obtain compounds of a desired profile.

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