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5-Ureidobenzofuranone indoles as potent and efficacious inhibitors of PI3 kinase-α and mTOR for the treatment of breast cancer

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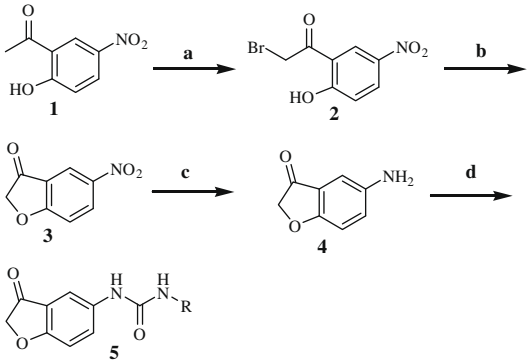
ABSTRACT

A series of 5-ureidobenzofuran-3-one indoles as potent inhibitors of PI3Kα and mTOR has been developed. The best potency in cells was obtained when the urea group was extended to a 4-[2-(dimethylamino)ethyl]methylamino amidophenyl group. A 7-fluoro group on the indole ring also enhanced cellular potency. Compound **18i**, incorporating the optimal functional groups, showed high potency in cellular lines and was further studied in vivo. It was able to inhibit the biomarker phosphorylation for 8 h when dosed at 25 mg/kg iv. In the MDA-MB-361 breast cancer model, it shrank the tumor size remarkably when dosed at 25 mg/kg iv on days 1, 5, and 9.

The phosphoinositol 3-kinase (PI3K) signaling pathway plays an important role in mitogenic signaling, cell survival, cell growth, proliferation, and metabolic control. PI3Ks (α, β, γ, δ) are a family of enzymes, which phosphorylate the 3′-OH position of the inositol ring of phosphatidylinositol-4,5-bisphosphate (PIP2).[1–3] Activation of the PI3K signaling cascade has a positive effect on cell growth, survival, and proliferation. Constitutive up-regulation of PI3K signaling is observed in several types of cancers, and can have a deleterious effect on cells, leading to uncontrolled proliferation, enhanced migration, and adhesion-independent growth. These events favor not only the formation of malignant tumors, but also development of inflammatory and autoimmune diseases.[1–3] The mammalian target of rapamycin (mTOR) is a key component of the PI3K signaling pathway.[4,5] It shares high sequence similarity with PI3K at the ATP binding site. Therefore, blocking the PI3K/AKT signaling pathway by inhibiting the lipid kinase PI3Kα (which phosphorylates PIP2 to PIP3) and/or mTOR provides a promising new strategy for cancer therapy. Active-site dual inhibitors of PI3K/mTOR have the potential for enhanced efficacy.[5,6]

We recently reported a series of 4,6-dihydroxybenzofuran-3-one indoles as inhibitors of PI3Kα and mTOR.[7] This work was further extended to hydroxybenzofuran-3-one 7-azaindoles[8] and ureidobenzofuran-3-one 7-azaindoles[9] as selective inhibitors of mTOR. As indicated by modeling based on the PI3Kγ crystal structure, the ureido moiety can mimic the hydroxyl binding interactions in the active site and capture further interaction common to both PI3K and mTOR binding. It has the advantage that it is not subject to glucuronidation as observed in the hydroxyl series, thus affording the compound higher metabolic stability. We now report a series of 5-ureidobenzofuran-3-one indoles as potent inhibitors of PI3Kα and mTOR.

The synthesis of 1-(3-oxo-2,3-dihydro-1-benzofuran-5-yl)urea (**5**) is shown in Scheme 1. Starting from 2-hydroxy-5-nitroaceto-



Scheme 1. Synthesis of 1-(3-oxo-2,3-dihydro-1-benzofuran-5-yl)urea (**5**). Reagents and conditions: (a) $CuBr_2$; (b) Et_3N; (c) H_2/Pd–C; (d) RN = C=O.

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phenone (**1**), bromination with $CuBr_2$ followed by cyclization led to 5-nitro-1-benzofuran-3(2*H*)-one (**3**). Reduction of the nitro group gave 5-amino-1-benzofuran-3(2*H*)-one (**4**).[9] Treatment with methylisocyanate or 3-pyridylisocyanate gave the desired intermediate **5**.

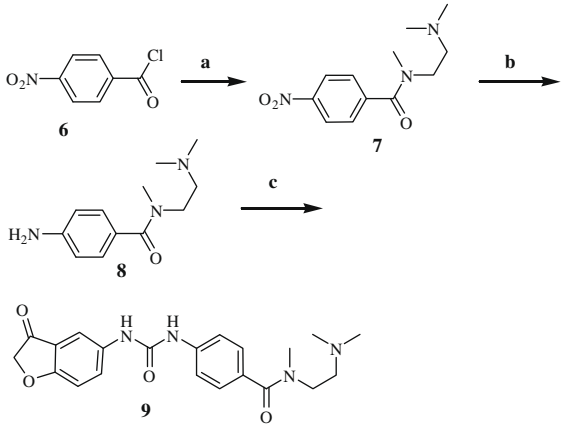
For more complex ureas where corresponding isocyanates are unavailable, the synthesis is exemplified in Scheme 2. Starting from 4-nitrobenzoyl chloride, amidation followed by hydrogenation gave 4-amino-*N*-[2-(dimethylamino)ethyl]-*N*-methylbenzamide (**8**), which was coupled with 5-amino-1-benzofuran-3(2*H*)-one (**4**) using triphosgene to give the desired 5-ureidobenzofuranone **9**.

Synthesis of the indole portion of the 7-fluoro compounds and final coupling is shown in Scheme 3. Des-fluoro compounds were prepared in a similar manner. Methylation of 3-fluoro-4-nitrophenol, followed by reduction of the nitro group gave compound **12**. A Gassman oxindole synthesis[10] provided compound **14**. The thiomethyl group was removed by Zn–Cu couple. Treatment with $POBr_3$ in DMF introduced both the formyl group and the bromo group. Suzuki coupling gave the desired indole aldehyde **17**, which was condensed with 5-ureidobenzofuranones **5** or **9** to give the desired final compound **18**. A single (*Z*)-isomer was obtained exclusively for the final products.

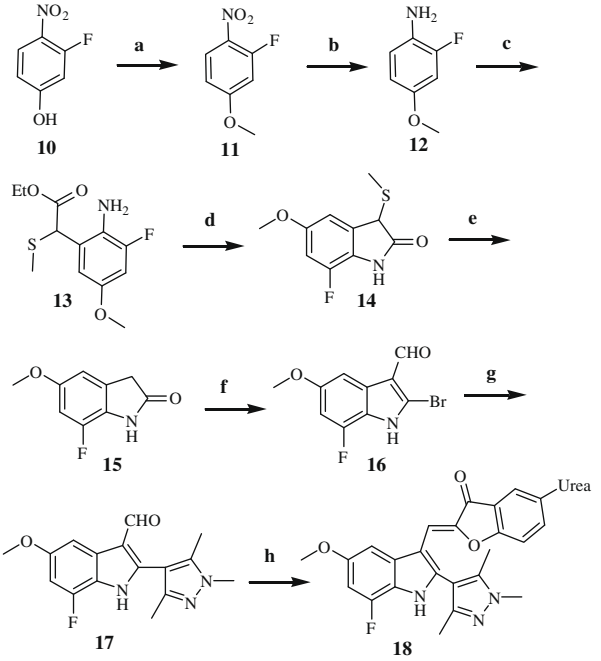
As shown in Table 1, compounds **18a–d** exhibited high potency in inhibiting both PI3Kα and mTOR. In the cellular assays, the 7-fluoro group on the indole ring afforded about twofold higher potency. Ureas **18b** and **18d**, bearing the 3-pyridyl group, were much less potent than the corresponding methyl ureas **18a** and **18c**, in the PC3-mm2 cells. Neither of these observations could be fully explained with solubility, or permeability data.

Excellent enzyme potency was retained with analogues **18e–i** (Table 2). Compound **18i** was shown to be a pan-PI3K inhibitor with IC_{50} values of 0.7, 2.4, and 0.6 nM versus PI3K β, γ, δ, respectively and was just as potent against two of the most frequently occurring mutant forms of PI3Kα (E545K and H1047R, 0.3 and 0.5 nM, respectively), as seen with our earlier morpholino-triazine based series bearing ureas.[11] Furthermore, these analogues with extended benzamido ureas achieved enhanced cellular potencies relative to **18a–d**, also as previously observed.[11,12]

Compound **19**, an analog of compound **18f** with a hydrogen instead of the 1,3,5-trimethyl-1*H*-pyrazol-4-yl group at the 2-position of the indole, achieved high selectivity for PI3Kα (IC_{50} = 0.9 nM) versus mTOR (IC_{50} = >4000 nM). However, its cellular potency was much lower than that of compound **18f**, underscoring the importance of the 1,3,5-trimethyl-1*H*-pyrazol-4-yl group at the 2-position for cellular activity. While lower cellular potency could be in part

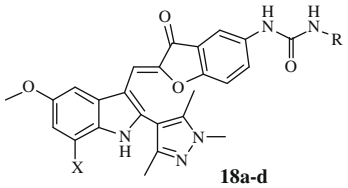


Scheme 2. Synthesis of *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-{[(3-oxo-2,3-dihydro-1-benzofuran-5-yl)carbamoyl]amino}benzamide (**9**). Reagents and conditions: (a) *N*,*N*,*N*′-Trimethylethylenediamine; (b) H_2/Pd–C; (c) triphosgene, then **4**.



Scheme 3. Synthesis of 1-[(2*Z*)-2-{[7-fluoro-5-methoxy-2-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-1*H*-indol-3-yl]methylidene}-3-oxo-2,3-dihydro-1-benzofuran-5-yl]urea (**18**). Reagents and conditions: (a) Me_2SO_4; (b) H_2/Pd–C; (c) ethyl 2-(methylthio)acetate, sulfuryl chloride; iPr_2NEt; (d) 0.1 N HCl; (e) Zn–Cu couple, 70 °C; (f) $POBr_3$/DMF; (g) 1,3,5-trimethyl-1*H*-pyrazole-4-boronic acid pinacol ester, Pd(0); (h) **5** or **9**, HCl, EtOH, 60 °C.

Table 1
In vitro data for 5-ureidobenzofuranones **18a–18d**

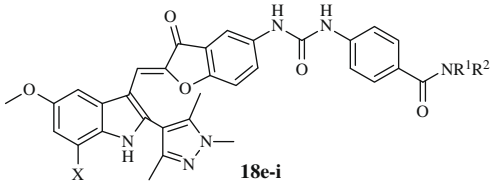


Compd	X	R	IC_{50}[a] (nM)			
			PI3Kα	mTOR	PC3-mm2	MDA-MB-361
18a	H	Methyl	0.4	1.3	130	36
18b	H	3-Pyridyl	0.5	5.2	>10,000	76
18c	F	Methyl	0.4	2.4	62	20
18d	F	3-Pyridyl	1.0	1.5	>1000	39

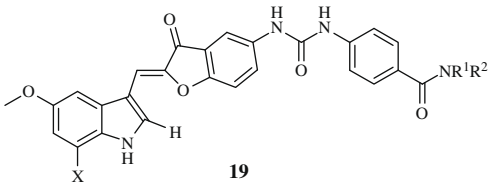
[a] Determinations were made at eight concentrations, in triplicate, and repeat values agreed within ⩽30%, on average.

due to lack of mTOR activity, this does not explain the total lack of activity observed in the two cell lines. Among various amino groups introduced, the highest cellular potency was obtained with [2-(dimethylamino)ethyl]methylamino group (compounds **18g** and **18i**). Compound **18i**, bearing the 7-fluoro group on the indole ring, provided the highest potency in the PC3-mm2 breast cell line and excellent potency in the MDA-MB-361 breast line, in part owing to the combined effect of enhanced mTOR and PI3K isoform potency. In vitro biomarker studies (Western blots) at 4 h post dosing in cells corroborated the potencies observed in the cell growth assays. Select compounds that were poor in PC3-mm2 growth inhibition were also tested for downstream effectors of PI3K. It appeared that the compounds could not reach the target in sufficient concentrations to inhibit AKT phosphorylation, owing in part to differences in permeability or efflux mechanisms in this cell line. As above, the

Table 2
In vitro data for 5-ureidobenzofuranones **18e–18i** and **19**



18e-i



19

Compd	X	NR^1R^2	IC_{50}[a] (nM)			
			PI3Kα	mTOR	PC3-mm2	MDA-MB-361
18e	H	4-Morpholinyl	0.2	4.2	94	6
18f	H	4-Methylpiperazin-1-yl	0.2	3.8	131	8
18g	H	[2-(Dimethylamino)ethyl]methylamino	0.3	1.1	23	<3
18h	F	4-Morpholinyl	0.5	2.5	57	6
18i	F	[2-(Dimethylamino)ethyl]methylamino	0.2	0.3	10	<3
19	H	4-Methylpiperazin-1-yl	0.9	>4000	>1000	>1000

[a] Determinations were made at eight concentrations, in triplicate, and repeat values agreed within ⩽30%, on average.

enhanced cellular potency of **18g**, and especially **18i**, could not be explained based only on permeability, efflux, or solubility since all the analogues were similar in these properties.

The predicted binding mode of **18g** in a PI3Kα homology model based on a PI3Kγ crystal structure (PDB access code 3MJW) is shown in Figure 1. The methoxy-group interacts with the hinge region backbone of Val851 and the urea makes three hydrogen bonding interactions with Asp810 and Lys802, which is the catalytic lysine. While good homology exists in the active site of PI3Kα, PI3Kγ, and mTOR, the rest of the model has poor homology and cannot explain the selectivity observed with **19** versus **18g**, or the other non-selective analogues bearing the bulky pyrazolyl group. The larger group appears to provide new interactions or a conformation (not achieved by **19**) that is more favorable for binding to mTOR, without affecting interactions with PI3Kα.

Compound **18i** had low water solubility (1 g/mL) at pH 7.4, however, it could be easily formulated at lower pH. Despite its low permeability (0.3×10^{-6} cm/s in both a–b and b–a directions in Caco-2 assays), based on its high stability achieved in three species ($t_{1/2}$ >30 min in nude mouse, rat, and human microsomes) and its excellent in vitro potency versus PI3K and cells, **18i** was selected for further in vivo studies. In biomarker studies (Fig. 2), when dosed at 25 mg/kg iv in nude mice, it inhibited phosphorylation

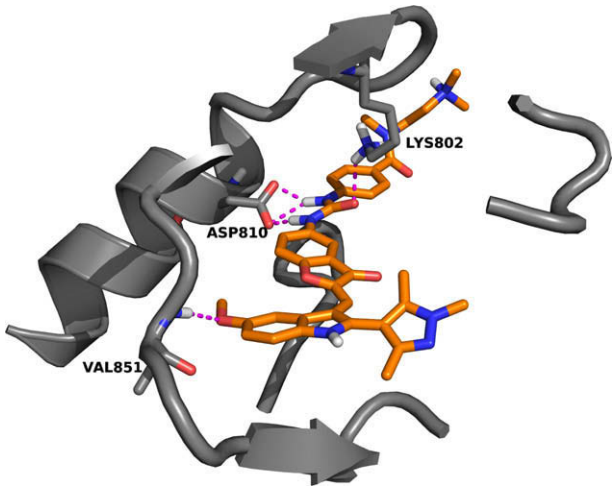


Figure 1. Close-up of the predicted binding mode of **18g** in a PI3Kα homology model based on the PI3Kγ crystal structure. Hydrogen bonding interactions are shown with magenta dashed lines.

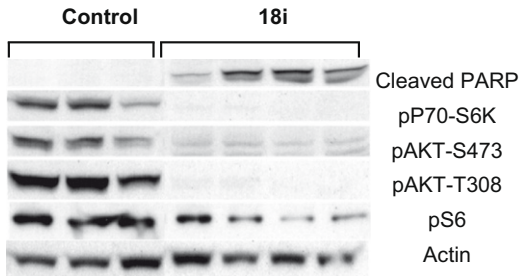


Figure 2. Biomarker inhibition by compound **18i** after 8 h, dosed in nude mice at 25 mg/kg iv.

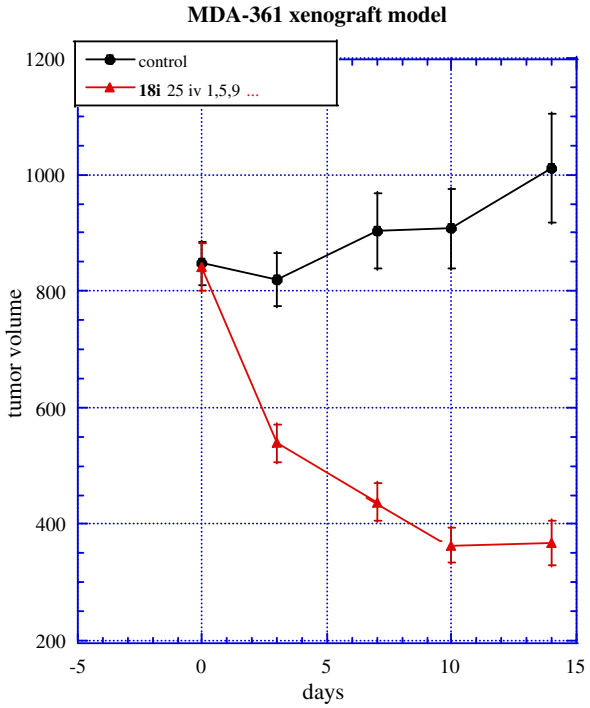


Figure 3. Tumor suppression by **18i,** dosed in nude mice at 25 mg/kg iv on days 1, 5, and 9.

of AKT-T308 (PI3K specific marker), AKT-S473, P70S6K, and PS6 (mTOR specific markers) after 8 h. It also induced cleaved poly ADP-ribose polymerase (PARP), a biomarker for cells undergoing

apoptosis. In the MDA-MB-361 xenograft model, **18i** was able to shrink the tumor size remarkably when dosed in nude mice at 25 mg/kg iv on days 1, 5, and 9 (Fig. 3).

In summary, we have developed a series of novel 5-ureidobenzofuran-3-one indoles as potent inhibitors of PI3Kα and mTOR. The best potency in cells was obtained when the urea group was extended with a 4-[2-(dimethylamino)ethyl]methylamino amidophenyl group. A 7-fluoro group on the indole ring also enhanced cellular potency. Compound **18i**, incorporating the optimal functional groups, showed high potency in cells and was further studied in vivo. It was able to inhibit biomarker phosphorylation for at least 8 h when dosed at 25 mg/kg iv. In the MDA-MB-361 in vivo efficacy model, it achieved remarkable shrinkage of a staged tumor with intermittent dosing at 25 mg/kg iv on days 1, 5, and 9.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.139.

References and notes

1. Liu, P.; Cheng, H.; Roberts, T. M.; Zhao, J. J. *Nat. Rev. Drug Disc.* **2009**, *8*, 627.
2. Carnero, A. *Expert Opin. Invest. Drugs* **2009**, *18*, 1265.
3. For review of PI3K inhibitors in oncology, see Verheijen, J. C.; Zask, A. *Drugs Future* **2007**, *32*, 537.
4. For review of mTOR inhibitors in oncology, see Verheijen, J. C.; Yu, K.; Zask, A. *Ann. Rep. Med. Chem.* **2008**, *43*, 189.
5. Feldman, M. E.; Apsel, B.; Uotila, A.; Loewith, R.; Knight, Z. A.; Ruggero, D.; Shokat, K. M. *PLoS Biol.* **2009**, *7*, 371.
6. Wang, X.; Yue, P.; Kim, Y. A.; Fu, H.; Khuri, F. R.; Sun, S.-Y. *Drugs Future* **2008**, *68*, 7409.
7. Bursavich, M. G.; Brooijmans, N.; Feldberg, L.; Hollander, I.; Kim, S.; Lombardi, S.; Park, K.; Mallon, R.; Gilbert, A. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2586.
8. Tsou, H.-R.; MacEwan, G.; Birnberg, G.; Grosu, G.; Bursavich, M. G.; Bard, J.; Brooijmans, N.; Toral-Barza, L.; Hollander, I.; Mansour, T. S.; Ayral-Kaloustian, S.; Yu, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2321.
9. Tsou, H.-R.; MacEwan, G.; Birnberg, G.; Zhang, N.; Brooijmans, N.; Toral-Barza, L.; Hollander, I.; Ayral-Kaloustian, S.; Yu, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2259.
10. Johnson, P. D.; Aristoff, P. A. *J. Org. Chem.* **1990**, *55*, 1374.
11. Venkatesan, A. M.; Dehnhardt, C. M.; Delos Santos, E.; Chen, Z.; Dos Santos, O.; Ayral-Kaloustian, S.; Khafizova, G.; Brooijmans, N.; Mallon, R.; Hollander, I.; Feldberg, L.; Lucas, J.; Yu, K.; Gibbons, J.; Abraham, R. T.; Chaudhary, I.; Mansour, T. S. *J. Med. Chem.* **2010**, *53*, 2636.
12. Dehnhardt, C. M.; Venkatesan, A. M.; Delos Santos, E.; Chen, Z.; Santos, O.; Ayral-Kaloustian, S.; Brooijmans, N.; Mallon, R.; Hollander, I.; Feldberg, L.; Lucas, J.; Chaudhary, I.; Yu, K.; Gibbons, J.; Abraham, R.; Mansour, T. S. *J. Med. Chem.* **2010**, *53*, 798.