Identification of a Novel Oxadiazole Inhibitor of Mammalian Target of Rapamycin

Sunwoo Lim,[†,‡,¶] Hyomin Lee,[‡,§,¶] Euijung Kim,[†,‡] and Wooyoung Hur[‡,§,*]

[†]*Department of Chemistry, Korea University, Seoul 02841, South Korea*
[‡]*Chemical Kinomics Research Center, Korea Institute of Science and Technology (KIST), 5 Hwarangro-14-gil, Seoul 02792, South Korea*
[§]*University of Science and Technology (UST), Daejeon 34113, South Korea. *E-mail: whur@kist.re.kr*
[¶]*These authors contributed equally to this work.*
Received November 6, 2019, Accepted December 19, 2019

We performed a biochemical screen against mTOR using in-house small molecule library. Two novel, structurally distinct hits were identified. Among them, a novel oxadiazole scaffold compound (**2**) suppressed the phosphorylation of both S6K1 and Akt1 in HeLa cells. Docking study suggested that **2** is ATP-competitive and shows a pi-pi interaction with Trp2239 and hydrogen bonds with Trp2239 and Thr2245. Through derivatization, a slightly more potent analogue (**2a**) was identified with IC_{50} of 9.6 μM. Our study provides a starting point for discovery of novel potent mTOR inhibitors.

Keywords: Mammalian target of rapamycin, mTOR, Inhibitor, Screening, 2,3-Dihydro-1,3,4-oxadiazole

Introduction

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is convergence point for regulation of a wide array of processes including energy homeostasis and cell growth.[1,2] mTOR exists in two distinct, but evolutionarily conserved mTOR complexes: mTORC1 (mTOR Complex **1**) and mTORC2 (mTOR Complex **2**). Both complexes share multiple components including mTOR, mLST8, Deptor, Tti1, and Tel2.[3–5] In addition, mTORC1 contains Raptor and PRAS40, while mTORC2 has Rictor, Protor, and mSin1 as specific constituents. Each complex is differentially regulated, differentially sensitive to rapamycin, and has distinct substrate specificity.

mTORC1 integrates signals from growth factors, cellular stresses, and nutrients such as amino acids, and induces protein translation through phosphorylation of S6K1 (Thr389) and 4EBP1, as well as the lipid synthesis by upregulating SREBP1 and PPARγ.[6] Also, mTORC1 suppresses autophagy through phosphorylation and inhibition of the ULK1.[7] The natural product mTORC1 inhibitor Rapamycin forms a complex with FK506-binding protein 12 (FKBP12), which binds to the FRB domain of mTOR and disrupts the mTORC1 complex formation.

Upon growth factor stimulation, mTORC2 is recruited to PIP_3 in the plasma membrane through the PH domain of mSin1, and directly phosphorylates Ser473 of Akt1, promoting cell proliferation and survival.[2] The mTORC2-Akt pathway is also responsible for the chaperone-mediated autophagy in lysosome.[8] Similarly, mTORC2 also phosphorylates the hydrophobic motif (Ser422) of another SGC family kinase SGK1,[9] but its physiological functions are still being uncovered. mTORC2 is inhibited by a long-term treatment of rapamycin, but the inhibition is cell-type dependent.[10]

mTOR is an important therapeutic target. Aberrantly activated mTOR is implicated in various diseases including cancers, diabetes, and neurodegenerative disorders.[1,11,12] Activation of mTOR pathway is also a major compensatory mechanism that causes resistance to targeted cancer therapy.[13] Rapamycin analogues such as Temsirolimus and Everolimus were FDA approved as anti-cancer drugs. However, their efficacy is limited by the negative feedback mechanism induced by the selective mTORC1 inhibition.[14] Targeting the ATP pocket of mTOR is a therapeutically more effective approach, because it ablates the functions of the both mTOR complexes. Numerous ATP-competitive mTOR inhibitors have been identified (Figure 1), many of which are in clinical evaluation for cancers.[15] Herein, we wish to report a novel scaffold mTOR inhibitor identified from a small molecule screen.

Results and Discussion

A high-throughput screening against recombinant human mTOR (amino acid 1362-end) was performed using ca. 2000 in-house compound library at a concentration of 30 μM. Time-resolved FRET (fluorescence resonance energy transfer) assay[16] was employed for the enzyme-based screening, which measured FRET signal between the terbium-labeled phospho-specific 4EBP1 antibody and the GFP-labeled 4EBP1 that is phosphorylated during mTOR kinase reaction. We excluded known mTOR inhibitors and reactive compounds, then identified two hits (**1**, **2**) (Figure 2). Compound **1** and **2** inhibited human mTOR *in vitro* with IC_{50}'s of 54 and 13 μM, respectively (Tables 1 and 2).

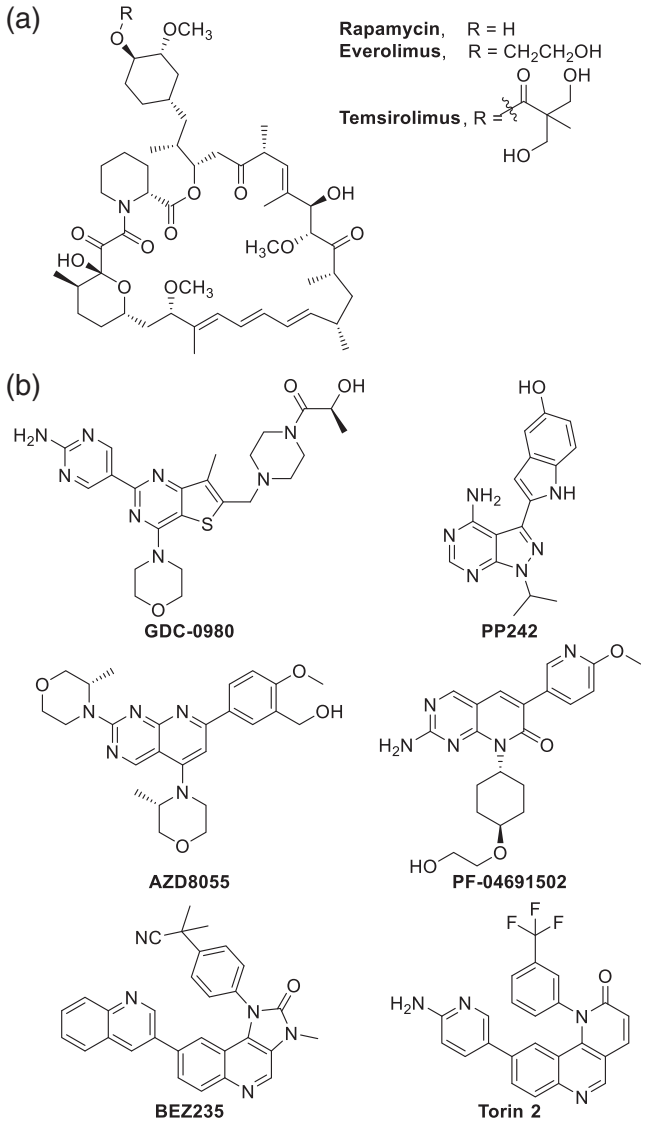


Figure 1 Structures of representative mTOR inhibitors. Structures of (a) rapalogues, (b) ATP-competitive inhibitors.

To evaluate the ability of **1** and **2** to inhibit mTOR in cells, each compound was treated to HeLa cells at 10 and 100 μM for 1 h, and the phosphorylation levels of S6K1 (Thr389) and Akt1 (Ser473) were examined (Figure 3).

Compound **1** enhanced the phosphorylation of both S6K1 and Akt1 in cells. The unexpected upregulation of mTOR downstream signaling may be due to its off-target effects. On the other hand, compound **2** suppressed both p-S6K1 and p-Akt1, indicating its inhibition of mTOR activity in cells.

We then prepared analogues of the two hit compounds and measured their IC_{50}'s. Analogues of **1** and **2** were synthesized following Schemes 1,2 and 3, respectively.

We predicted that two hydrogen bonds between the pyrazole moiety of **1** and G2238, V2240 at the hinge backbone and a hydrogen bond between *p*-OH group at the trihydroxyphenyl moiety and Cys2243. Substitution of the tri-hydroxyphenyl group in **1** with *p*-hydroxy (**1a**-**c**) or *o*, *p*-dihydroxy (**1d**, **1e**) phenyl groups decreased the inhibitory activity (Table 1), indicating that the predicted hydrogen bond between *p*-OH group and Cys2243 does not exist, and instead the *m*-OH group may be involved in the binding. Also, the phenoxy group of **1** was predicted to be positioned as the aminopyridine ring of Torin2. Thus we introduced *p*-NH_2 group at the phenoxy group, expecting a potential hydrogen bond with the side chain of Asp2195 as shown in Torin2-mTOR interaction. However, this attempt (**1b**, **1d–f**) also failed to improve the inhibitory activity, suggesting that our predicted binding model was incorrect.

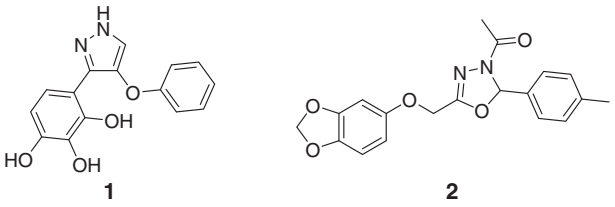
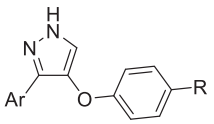


Figure 2 Structures of two hit compounds identified from an enzymatic mTOR inhibitor screen.

To investigate the structure–activity relationship (SAR) of **2**, we first varied the *p*-tolyl moiety. We found that *o*-tolyl analogue (**2a**) showed a slightly better inhibition (IC_{50} = 9.6 μM), while *m*-chlorophenyl (**2b**) and

Table 1. *in vitro* mTOR inhibition by analogues of **1.**



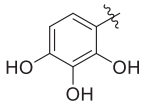
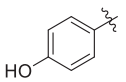
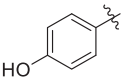
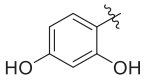
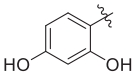
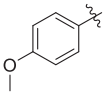
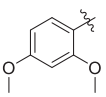
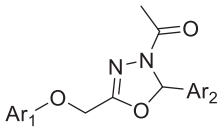
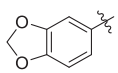
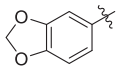
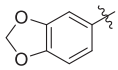
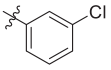
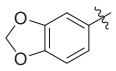
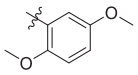
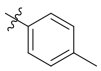
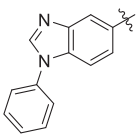
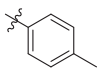
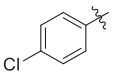
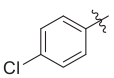
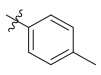
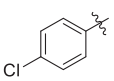
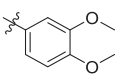
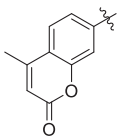
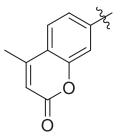
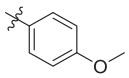
Compound	Ar	R	IC_{50} (μM)
1		H	54
1a		H	>100
1b		NH_2	>100
1c		H	>100
1d		NH_2	>100
1e		NH_2	>100
1f		NH_2	>100

Table 2. *in vitro* mTOR inhibition by analogues of **2**.



Compound	Ar_1	Ar_2	IC_{50} (μM)
2			13
2a			9.6
2b			31
2c			>100
2d			>100
2e			>100
2f			>100
2g			>100
2h			>100
2i			>100
2j			>100

2,5-dimethoxy phenyl derivatives showed a lower (IC_{50} = 31 μM) and no inhibition, respectively (Table 2).

In addition, replacing the benzodioxole moiety with phenyl group (**2d**) removed the inhibitory activity, suggesting that the dioxolane ring in the benzodioxole moiety makes an important contact with mTOR. We also introduced *para*-chlorophenyl (**2f**-**2h**) or coumarin (**2i, 2j**) at Ar_1 and derivatized Ar_2, but all of them showed IC_{50} > 100 μM.

We performed a docking study for **2** against the mTOR active site based on the reported X-ray crystal structure (Figure 4).[17] The benzodioxole moiety is predicted to be positioned for π–π interaction with the side chain of Trp2239 within the hinge region, and an oxygen atom of the benzodioxole forms a hydrogen bond with the amide backbone NH of Val2240. The carbonyl oxygen of **2** is located for a hydrogen bond with the side chain of Thr2245.

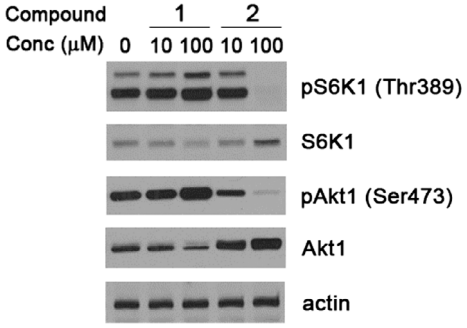


Figure 3 Western blot analysis for phospho-levels of S6K1 (Thr389) and Akt1 (Ser473) in HeLa cells following treatment of each compound at 10, 100 μM for 1 h.

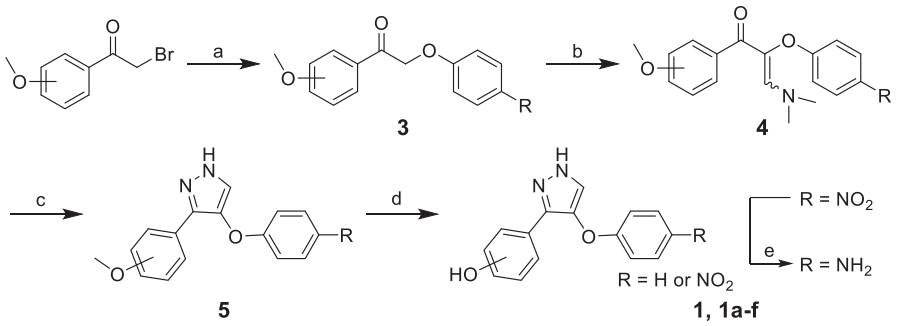
The toluene group is positioned for hydrophobic interaction with the side chain of Trp2239. This prediction model was consistent with the SAR result described in Table 2. For example, the bulky phenyl group attached to benzo[*d*] imidazole moiety of **2e** created a steric clash within the hinge region. The oxygen in the coumarin group of **2j** and **2k** did not fit for the hydrogen bond with the hinge region. Thus the benzodioxole moiety may not provide enough rooms for derivatization, and instead the *o*-tolyl moiety of **2a** is a better modification spot to enhance activity. The *ortho*-methyl of the toluene moiety could be elaborated and optimized for additional hydrophobic group that allows for new interactions with the hydrophobic surface area comprised of F2182, V2183, F2184, and L2185.

Conclusion

We identified two structurally distinct mTOR inhibitors from in-house small molecule library screen. Among the two hits, a novel oxadiazole scaffold compound **2** was able to suppress the phosphorylations of mTORC1/2 substrates (S6K1, Akt1) in cells. Docking study suggested that **2** binds in the ATP-binding pocket of mTOR. Through derivatization of **2**, we identified more potent analogue (**2a**) that inhibits mTOR with IC_{50} of 9.6 μM. Our results could be elaborated to a discovery of new potent mTOR inhibitors.

Experimental

General Information for Synthesis. Unless otherwise described, all commercial reagents and solvents were purchased from commercial suppliers and used without further purification. All reactions were performed under N_2 atmosphere in flame-dried glassware. Structures of synthesized compounds were confirmed by the ^1H NMR spectra using



Reagents and conditions: a. ArOH, K_2CO_3, DMF, rt, 4 h, 87-95%; b. *N*,*N*-dimethyl-formamide dimethyl acetal, toluene, 100 °C, 20 h, 81-96%; c. hydrazine, EtOH, 65 °C, 4 h, 14-45%; d. BBr_3, CH_2Cl_2, -78 °C -> rt, 3 h, 11-56%; e. Pd/C, H_2, MeOH, rt, 4 h, 20-78%.

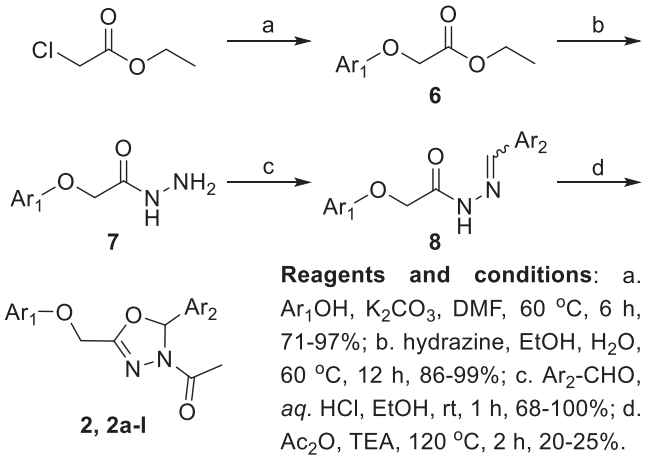
Scheme 1 Synthesis of derivatives of compound 1.

a Bruker 400 MHz FT-NMR (Billerica, MA, USA). The reaction progress was checked on precoated TLC Silica gel 60 F_{254} glass plates from Merck (Darmstadt, Germany) under UV light (254 nm). Silica gel (Kieselgel 60 Art. 9385, 230–400 mesh) was used for column chromatography.

Representative Synthesis (Compound 1d**) for Analogues of 1**

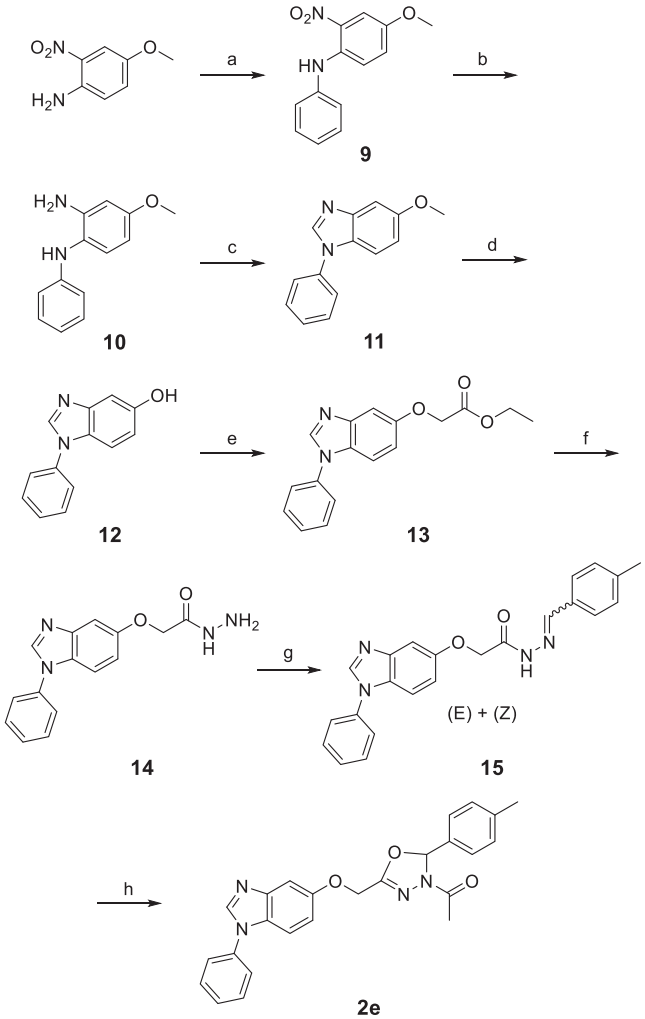
1-(2,4-Dimethoxyphenyl)-2-(4-nitrophenoxy)ethan-1-one (3). 2-Bromo-1-(2,4-dimethoxyphenyl)ethan-1-one (5 g) was added to a mixture of 4-nitrophenol (3.34 g, 24.0 mmol) and K_2CO_3 (6.03 g, 43.7 mmol) dissolved in DMF (44 mL), and the solution was stirred at room temperature for 4 h. The mixture was transferred into a flask filled with ice water (30 mL) and stirred at room temperature for 30 min. The solids were filtered to afford the corresponding product (87–94% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22–8.11 (m, 2H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.13–7.01 (m, 2H), 6.73 (d, *J* = 2.3 Hz, 1H), 6.69 (dd, *J* = 8.8, 2.3 Hz, 1H), 5.45 (s, 2H), 3.97 (s, 3H), 3.88 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 318.0978. Found: 318.0966.

1-(2,4-Dimethoxyphenyl)-3-(dimethylamino)-2-(4-nitrophenoxy)prop-2-en-1-one (4). *N*,*N*-dimethylformamide dimethyl acetal (8.88 mL, 66.9 mmol) was added to a solution of **3** (5.40 g, 22.3) dissolved in toluene (32 mL). The mixture was stirred and refluxed for 20 h, then cooled down to room temperature. The reaction solution was stirred at room temperature for 1 h until yellow



Reagents and conditions: a. Ar_1OH, K_2CO_3, DMF, 60 °C, 6 h, 71-97%; b. hydrazine, EtOH, H_2O, 60 °C, 12 h, 86-99%; c. Ar_2-CHO, *aq.* HCl, EtOH, rt, 1 h, 68-100%; d. Ac_2O, TEA, 120 °C, 2 h, 20-25%.

Scheme 2 Synthesis of derivatives of compound 2.



Reagents and conditions: a. Iodobenzene, CuI, K_2CO_3, DMF, 125 °C, 24 h, 81%; b. Pd/C, H_2, MeOH, rt, 5 h, 97%; c. formamidine acetate, 2-methoxyethanol, 80 °C, 8 h, 96%; d. BBr_3, DCM, 0 °C -> rt, 4 h, 66%; e. ethyl chloroacetate, K_2CO_3, DMF, 60 °C, 6 h, 71%; f. hydrazine, EtOH, H_2O, 60 °C, 12 h, 66%; g. 4-methylbenzaldehyde, *aq.* AcOH, EtOH, rt, 1 h, 66%; h. Ac_2O, DIPEA, 120 °C, 2 h, 20%.

Scheme 3 Synthesis of 2e.

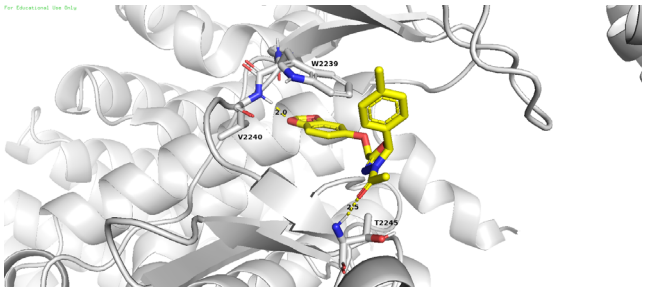


Figure 4. Docking result for 2 (yellow) based on mTOR crystal structure (PDB id: 4jsx). Three interacting residues in the ATP binding pocket are labeled and two hydrogen bonds are noted in yellow dotted lines.

solids were formed, and was filtered and washed with *n*-hexane to afford the desired product as a yellow powder (81–96% yield). ^{1}H NMR (400 MHz, Choroform-d) δ 8.19 (d, *J* = 9.1 Hz, 2H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 2H), 6.81 (s, 1H), 6.59–6.39 (m, 2H), 3.83 (s, 6H), 3.00 (s, 6H). HRMS (ESI) *m/z* calculated for $C_{19}H_{21}N_2O_6^+$ $[M + H]^+$: 373.1400. Found: 373.1390.

***3-(2,4-Dimethoxyphenyl)-4-(4-Nitrophenoxy)-1H*-Pyrazole (5).** Hydrazine monohydrate (8.64 mL, 178.2 mmol) was added to a stirring solution of **4** (6.10 g, 17.8 mmol) in ethanol (36 mL), and stirred at 65°C for 4 h. The reaction mixture was cooled down to room temperature, and extracted with isopropanol: chloroform (1:4) and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The crude material was then purified by flash chromatography (CH_2Cl_2: MeOH 24:1 v/v) to afford the desired product (14–45% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 1H), 7.60 (s, 1H), 7.10 (d, *J* = 8.9 Hz, 2H), 6.55 (s, 1H), 6.49 (d, *J* = 8.9 Hz, 1H), 3.96 (s, 3H), 3.81 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{17}H_{16}N_3O_5^+$ $[M + H]^+$: 342.1090. Found: 342.1079.

***4-(4-(4-Nitrophenoxy)-1H*-pyrazol-3-yl)benzene-1,3-diol.** A mixture of **5** (770 mg, 2.3 mmol) and anhydrous CH_2Cl_2 was cooled down to −78 °C, and 1.0 M boron tribromide in CH_2Cl_2 (11.3 mL, 11.3 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h, then quenched with ice-cold water and saturated aqueous $NaHCO_3$. The reaction mixture was extracted with isopropanol: chloroform (1:4), and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and was purified by flash chromatography (CH_2Cl_2:THF 6:1 v/v) to yield the corresponding product (11–56% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, *J* = 8.7 Hz, 1H), 6.68 (d, *J* = 2.3 Hz, 1H), 6.65 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.71 (s, 2H), 3.92 (s, 3H), 3.86 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{17}H_{18}N_3O_3^+$ $[M + H]^+$: 312.1348. Found: 312.1348.

***4-(4-(4-Aminophenoxy)-1H*-pyrazol-3-yl)benzene-1,3-diol (1d).** To a suspension of Pd/C (10% wt, Pd in charcoal) in MeOH (7 mL) was added the nitro compound (400 mg, 1.3 mmol). The mixture was hydrogenated under H_2 atmosphere (1 atm, balloon) at room temperature for 4 h. The reaction mixture was poured through Celite cake filter, washed with methanol, and the filtrate was concentrated *in vacuo*. The mixture was then washed with CH_2Cl_2: MeOH (9:1 v/v) to afford corresponding amino compound, **1d** (20–78% yield). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.09 (s, 1H), 11.01 (s, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 7.58 (s, 1H), 6.96–6.72 (m, 2H), 6.71–6.59 (m, 2H), 6.40 (d, *J* = 2.5 Hz, 1H), 6.33 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.44 (s, 2H). HRMS (ESI) *m/z* calculated for $C_{15}H_{14}N_3O_3^+$ $[M + H]^+$: 284.1035. Found: 284.1026.

Representative Synthesis (Compound 2) for Analogues of 2 (2, 2a–d, 2f–j)

***Ethyl* 2-(*benzo[d]*[1,3]dioxol-5-yloxy)acetate (6).** To a solution of benzo[d][1,3]dioxol-5-ol (1.00 g, 7.2 mmol) in DMF (14 mL) was added K_2CO_3 (2.00 g, 14.48 mmol), and was stirred at room temperature for 10 min. Ethyl chloroacetate (0.81 mL, 7.602 mmol) was added, and the reaction mixture was stirred for 6 h at 60 °C. The mixture was cooled down to room temperature and quenched with water, and the solution was extracted twice with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography (*n*-hexane:EtOAc 3:1 v/v) to afford pale yellow solid (71–97% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 6.69 (d, *J* = 8.5 Hz, 1H), 6.53 (d, *J* = 2.6 Hz, 1H), 6.31 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.92 (s, 2H), 4.54 (s, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H). HRMS (ESI) *m/z* calculated for $C_{11}H_{12}O_5Na^+$ $[M + Na]^+$:247.0582. Found: 247.0562.

***2-(Benzo[d]*[1,3]dioxol-5-yloxy)acetohydrazide (7).** To a solution of **6** (1.58 g, 7.0 mmol) in EtOH (18 mL) was added hydrazine monohydrate (3.45 mL, 70.5 mmol). The reaction mixture was stirred for 12 h at 60 °C, cooled down to room temperature, then EtOH was removed under reduced pressure. The crude compound was washed with *n*-hexane to obtain the desired product as white powder (86–99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.66 (d, *J* = 2.5 Hz, 1H), 6.38 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.96 (s, 2H), 4.39 (s, 2H), 4.31 (s, 2H). HRMS (ESI) *m/z* calculated for $C_9H_{10}N_2O_4Na^+$ $[M + Na]^+$: 233.0538. Found: 233.0517.

***2-(Benzo[d]*[1,3]dioxol-5-yloxy)-N′-(4-methylbenzylidene) acetohydrazide (8).** To a mixture of **7** (150 mg, 0.71 mmol) in EtOH (4 mL) was added 4-methylbenzaldehyde (0.09 mL, 0.79 mmol) and few drops of 37% aq. HCl solution. The reaction mixture was stirred for 1 h at room temperature, then quenched with ice water and saturated aqueous $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting solid was washed with Et_2O and CH_2Cl_2 (9:1 v/v) to afford white solid (68–100% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ

11.47 (s, 1H), 8.13 (d, *J* = 135.4 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 2H), 7.25 (t, *J* = 7.1 Hz, 2H), 6.81 (dd, *J* = 14.3, 8.5 Hz, 1H), 6.67 (dd, *J* = 26.2, 2.6 Hz, 1H), 6.39 (ddd, *J* = 28.3, 8.5, 2.6 Hz, 1H), 5.96 (d, *J* = 6.0 Hz, 2H), 4.80 (d, *J* = 188.3 Hz, 2H), 2.33 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{17}H_{17}N_2O_4^+$ $[M + H]^+$: 313.1188. Found: 313.1176.

***(5-((Benzo[d]*[1,3]dioxol-5-yloxy)methyl)-2-methyl-1,3,4-oxadiazol-3(2*H*)-yl)(p-tolyl)methanone** (2)**.** To a mixture of **8** (70 mg, 0.22 mmol) in acetic anhydride (2 mL) was added triethylamine (0.09 mL, 0.67 mmol), and was stirred for 2 h at 120 °C. The reaction mixture was cooled down to room temperature, and mixed with saturated aqueous $NaHCO_3$. The solution was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4. The crude compound was purified by flash chromatography (*n*-hexane:EtOAc = 6:1 v/v) to afford desired product **2** (20–25% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.56 (s, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.27 (d, *J* = 7.3 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 1H), 6.57 (d, *J* = 2.5 Hz, 1H), 6.36 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.92 (s, 2H), 5.09 (s, 2H), 2.53 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.77, 169.49, 165.11, 153.53, 148.33, 142.98, 142.37, 130.10, 129.86, 129.72, 129.69, 128.52, 107.93, 106.25, 101.28, 98.66, 69.95, 26.13, 21.69. HRMS (ESI) *m/z* calculated for $C_{19}H_{19}N_2O_5^+$ $[M + H]^+$: 355.1294. Found: 355.1290.

***1-(5-((Benzo[d]*[1,3]dioxol-5-yloxy)methyl)-2-(*o*-tolyl)-1,3,4-oxadiazol-3(2*H*)-Yl)ethan-1-one** (2a)**.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.25 (s, 1H), 7.74 (d, *J* = 7.0 Hz, 1H), 7.30–7.20 (m, 3H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.63 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.95 (s, 2H), 5.03 (s, 2H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 171.80, 169.45, 164.30, 153.51, 148.34, 142.40, 139.00, 131.88, 131.24, 127.60, 126.40, 107.93, 106.30, 101.30, 98.69, 70.10, 30.33, 26.18, 19.73. HRMS (ESI) *m/z* calculated for $C_{19}H_{19}N_2O_5^+$ $[M + H]^+$: 355.1294. Found: 355.1293.

Synthesis of Compound 2e

***4-Methoxy-2-nitro-N*-phenylaniline** (9)**.** To a solution of 4-methoxy-2-nitroaniline (5.00 g, 29.74 mmol) in DMF (59 mL) was added copper iodide (0.56 g, 2.97 mmol), K_2CO_3 (12.33 g, 89.21 mmol), and iodobenzene (8.09 mL, 59.47 mmol). The reaction mixture was stirred for 24 h at 125 °C, cooled down to room temperature, then copper was removed by passing the reaction mixture through a pad of Celite. The filtrate was extracted twice with CH_2Cl_2 and water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude compound was purified by flash chromatography (*n*-hexane:EtOAc = 93:7 v/v) to afford the desired product in a form of red oil (81% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.37 (s, 1H), 7.67 (d, *J* = 3.0 Hz, 1H), 7.42 (dd, *J* = 8.4, 7.3 Hz, 2H), 7.31–7.25 (m, 3H), 7.22 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.10 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.86 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 245.0926. Found: 245.0908.

***4-Methoxy-N*^{1}-Phenylbenzene-1,2-Diamine** (10)**.** To a suspension of Pd/C (0.58 g, 10% wt, Pd in charcoal) in MeOH (40 mL) was added **9** (5.76 g, 23.58 mmol). The mixture was hydrogenated under H_2 atmosphere (1 atm, balloon) at room temperature for 5 h. The reaction mixture was filtered through Celite and washed with methanol, and the filtrate was concentrated *in vacuo*, washed with *n*-hexane and CH_2Cl_2 (9:1 v/v) to afford gray solid as the corresponding amino compound (97% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.19 (t, *J* = 7.0 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.78 (td, *J* = 7.3, 1.5 Hz, 1H), 6.64 (dd, *J* = 7.5, 1.2 Hz, 2H), 6.38 (d, *J* = 2.7 Hz, 1H), 6.33 (ddd, *J* = 8.5, 2.8, 1.6 Hz, 1H), 5.03 (s, 1H), 3.88 (s, 2H), 3.79 (d, *J* = 1.2 Hz, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 215.1184. Found: 215.1183.

***5-Methoxy-1-Phenyl-1H*-Benzo[*d*]Imidazole** (11)**.** To a solution of **10** (4.00 g, 18.67 mmol) in 2-methoxyethanol (47 mL) was added formamidine acetate (2.92 g, 28.00 mmol). The solution was stirred and refluxed for 8 h at 80°C, cooled down to room temperature, then water was added, and the reaction mixture was extracted twice with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography (*n*-hexane:THF = 4:1 v/v) to afford red to orange oil product (96% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 7.71–7.57 (m, 4H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.52–7.44 (m, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 6.95 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.82 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 225.1028. Found: 225.1039.

***1-Phenyl-1H*-Benzo[*d*]Imidazol-5-Ol** (12)**.** A mixture of **11** (3.80 g, 2.3 mmol) and anhydrous CH_2Cl_2 (34 mL) was cooled down to −78 °C, and 1.0 M boron tribromide in CH_2Cl_2 (33.89 mL, 33.89 mmol) was slowly added. The mixture was stirred at room temperature for 4 h, then quenched with ice water and saturated aqueous $NaHCO_3$ at 0 °C. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and was purified by flash chromatography (CH_2Cl_2:MeOH = 20:1 v/v), and the resulting solid was washed once more with CH_2Cl_2 to yield the corresponding product (67% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.67 (s, 1H), 7.85–7.75 (m, 2H), 7.76–7.66 (m, 2H), 7.65 (dd, *J* = 7.3, 1.5 Hz, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 2.2 Hz, 1H), 7.08 (dd, *J* = 9.0, 2.3 Hz, 1H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 211.0871. Found: 211.0988.

***Ethyl 2-((1-Phenyl-1H*-Benzo[*d*]Imidazol-5-Yl)Oxy) Acetate** (13)**.** To a solution of **12** (1.90 g, 9.04 mmol) in DMF (23 mL) was added K_2CO_3 (2.50 g, 18.08 mmol), and was stirred at room temperature for 10 min. Ethyl chloroacetate (1.16 mL, 9.49 mmol) was added, and the reaction mixture

was stirred for 6 h at 60 °C. The mixture was cooled down to room temperature and quenched with water, and the solution was extracted twice with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography (*n*-hexane:THF = 2:1 v/v) and light beige solid was afforded as product (71% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (d, *J* = 2.7 Hz, 1H), 7.59–7.53 (m, 2H), 7.49 (ddd, *J* = 8.5, 3.7, 1.6 Hz, 2H), 7.44 (dt, *J* = 8.8, 2.5 Hz, 2H), 7.29 (d, *J* = 2.4 Hz, 1H), 7.07 (dq, *J* = 9.1, 2.3 Hz, 1H), 4.70 (s, 2H), 4.29 (qt, *J* = 7.2, 2.4 Hz, 2H), 1.31 (td, *J* = 7.2, 4.0 Hz, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 297.1239. Found: 297.1250.

***2-((1-Phenyl-1H*-benzo[*d*]imidazol-5-Yl)oxy) acetohydrazide (14).** To a solution of **13** (1.35 g, 4.56 mmol) in EtOH (13 mL) was added hydrazine monohydrate (2.21 mL, 45.56 mmol). The reaction mixture was stirred for 12 h at 60 °C, cooled down to room temperature, then EtOH was removed under reduced pressure. The crude compound was washed with *n*-hexane to obtain the desired product as white powder (86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.51 (s, 1H), 7.70–7.57 (m, 4H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.49 (tt, *J* = 7.2, 1.5 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 7.04 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.55 (s, 2H), 4.34 (s, 2H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 283.1195. Found: 283.1206.

***N*′-(4-Methylbenzylidene)-2-((1-phenyl-1*H*-benzo[*d*] imidazol-5-Yl)oxy)acetohydrazide (15).** To a mixture of **14** (1.00 g, 4.76 mmol) in EtOH (16 mL) was added 4-tolualdehyde (0.62 mL, 5.23 mmol) and few drops of acetic acid. The reaction mixture was stirred for 1 h at room temperature, then quenched with ice water and saturated aqueous $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting solid was washed with Et_2O and CH_2Cl_2 (5:1 v/v) to afford the corresponding product as a white solid (68% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.55 (s, 1H), 8.51 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 130.6 Hz, 1H), 7.75–7.51 (m, 7H), 7.51–7.46 (m, 1H), 7.39–7.20 (m, 3H), 7.05 (ddd, *J* = 30.3, 8.9, 2.5 Hz, 1H), 4.96 (d, *J* = 188.4 Hz, 2H), 2.34 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 385.1665. Found: 385.1674.

***1-(5-(((1-Phenyl-1H*-benzo[*d*]Imidazol-5-Yl)oxy) methyl)-2-(p-tolyl)-1,3,4-oxadiazol-3(2*H*)-Yl)ethan-1-one (2e).** To a mixture of **15** (50 mg, 0.13 mmol) in acetic anhydride (1 mL) was added DIPEA (0.07 mL, 0.67 mmol), and was stirred for 2 h at 120 °C. The reaction mixture was cooled down to room temperature, and mixed with saturated aqueous $NaHCO_3$. The solution was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4. The crude compound was purified by flash chromatography (*n*-hexane:EtOAc = 6:1 v/v) to afford desired product (20% yield). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.66 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.71–7.60 (m, 4H), 7.50 (dd, *J* = 8.5, 5.7 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.29 (d, *J* = 2.5 Hz, 1H), 7.05 (dd, *J* = 8.9, 2.5 Hz, 1H), 5.29 (s, 2H), 2.78 (s, 1H), 2.47 (s, 3H), 2.41 (s, 3H). HRMS (ESI) *m/z* calculated for $C_{16}H_{16}NO_6^+$ $[M + H]^+$: 427.1770. Found: 427.1785.

Biological Materials. HeLa cells were maintained in DMEM, 10% FBS (Sigma Aldrich, St.Louis, MO, USA) supplemented with penicillin/streptomycin. Antibodies against phospho-Akt1 (Ser473), Akt1, phospho-S6K1 (Thr389), and S6K1 were from Cell Signaling Technology (Danvers, MA, USA). The recombinant human mTOR (1362-end) protein was from Sigma Aldrich.

***in vitro* mTOR Kinase Assay.** *in vitro* mTOR activity was assayed using the Lanthascreen™ time-resolved FRET assay (Thermo Fisher Scientific, Waltham, MA, USA). Recombinant mTOR (3 nM) was incubated with serially diluted compounds (100 nL) for 30 min in 5 μL of kinase buffer (25 mM HEPES, pH 7.4, 8 mM $MgCl_2$, 6 mM $MnCl_2$, 4 mM DTT) in a 384-well low-volume white plate (Corning). Then an equal volume of the kinase buffer was added containing 0.6 μM GFP-4EBP1 and 20 μM ATP. After incubation at rt. for 90 min, 5 μL of stop solution containing 45 mM EDTA and 4.5 nM Tb-labeled phospho-4EBP1 (Thr46) antibody. After 30 min, the FRET signal (FL_{520}/FL_{495} ratio) between Tb and GFP was measured using Envision plate reader (PerkinElmer, Waltham, MA, USA). Each assay was duplicated and IC_{50} values were calculated using Prism7 software (GraphPad).

Docking Study. Crystal structure of mTOR (PDB: 4JSX) was retrieved from the Protein Data Bank. Molecular docking studies and molecular dynamics simulation studies of the mTOR complex with compound **2** were performed using Maestro and Pymol software.

Acknowledgments. This work was supported by Korea Institute of Science and Technology (2E30240).

References

1. M. Laplante, D. M. Sabatini, *Cell* **2012**, *149*, 274.
2. D. A. Guertin, D. M. Sabatini, *Cancer Cell* **2007**, *12*, 9.
3. T. Kaizuka, T. Hara, N. Oshiro, U. Kikkawa, K. Yonezawa, K. Takehana, S. Iemura, T. Natsume, N. Mizushima, *J. Biol. Chem.* **2010**, *285*, 20109.
4. T. R. Peterson, M. Laplante, C. C. Thoreen, Y. Sancak, S. A. Kang, W. M. Kuehl, N. S. Gray, D. M. Sabatini, *Cell* **2009**, *137*, 873.
5. D. H. Kim, D. D. Sarbassov, S. M. Ali, R. R. Latek, K. V. Guntur, H. Erdjument-Bromage, P. Tempst, D. M. Sabatini, *Mol. Cell* **2003**, *11*, 895.
6. M. Laplante, D. M. Sabatini, *J. Cell Sci.* **2013**, *126*, 1713.
7. J. Kim, M. Kundu, B. Viollet, K. L. Guan, *Nat. Cell Biol.* **2011**, *13*, 132.
8. E. Arias, H. Koga, A. Diaz, E. Mocholi, B. Patel, A. M. Cuervo, *Mol. Cell* **2015**, *59*, 270.
9. J. M. Garcia-Martinez, D. R. Alessi, *Biochem. J.* **2008**, *416*, 375.
10. D. D. Sarbassov, S. M. Ali, S. Sengupta, J. H. Sheen, P. P. Hsu, A. F. Bagley, A. L. Markhard, D. M. Sabatini, *Mol. Cell* **2006**, *22*, 159.

11. P. B. Crino, *Nat. Rev. Neurol.* **2016**, *12*, 379.
12. R. Zoncu, A. Efeyan, D. M. Sabatini, *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 21.
13. Y. Guri, M. N. Hall, *Trends Cancer* **2016**, *2*, 688.
14. K. E. O'Reilly, F. Rojo, Q. B. She, D. Solit, G. B. Mills, D. Smith, H. Lane, F. Hofmann, D. J. Hicklin, D. L. Ludwig, J. Baselga, N. Rosen, *Cancer Res.* **2006**, *66*, 1500.
15. D. Benjamin, M. Colombi, C. Moroni, M. N. Hall, *Nat. Rev. Drug Discov.* **2011**, *10*, 868.
16. E. Ekin, D. Arin, P. Mahmut, E. E. Ayse, M. E. Yasar, *Curr. Pharma. Biotech* **2016**, *17*, 1222.
17. H. Yang, D. G. Rudge, J. D. Koos, B. Vaidialingam, H. J. Yang, N. P. Pavletich, *Nature* **2013**, *497*, 217.

