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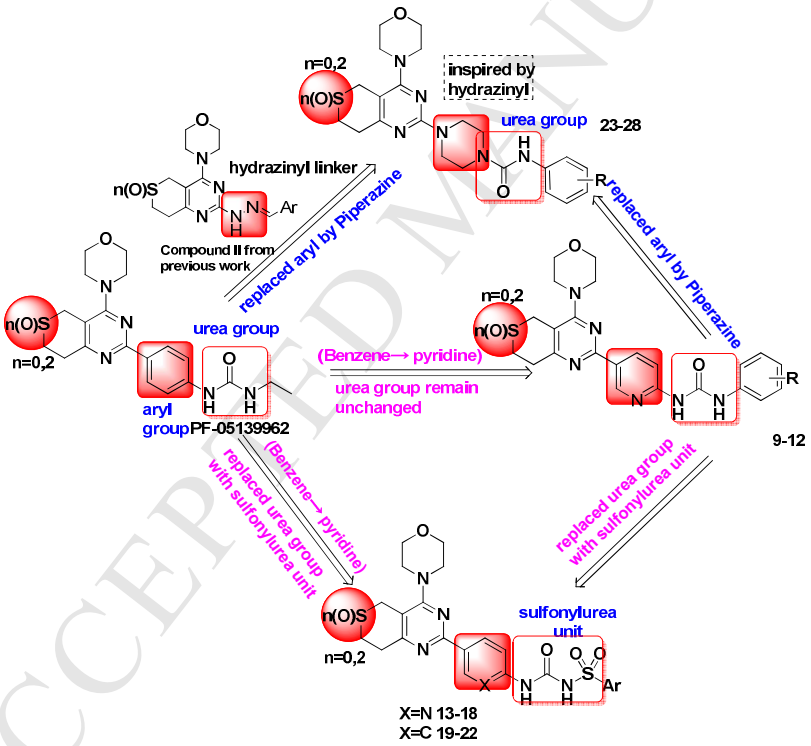
Graphical Abstract



Four series of 2-substituted-4-morpholino- 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives were synthesized and evaluated for their activity against PI3Kα and mTOR kinase

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and cancer cell lines. The most promising compound **11** showed good antitumor potency for A549, PC-3 and MCF-7 cell lines with IC50 values of 0.52 ± 0.10μM, 1.41 ± 0.10μM, and 4.82±0.24μM, and strong antitumor activities against PI3Kα/mTOR with IC_{50} values of 6.72 ± 0.30 μM and 0.94 ± 0.10 μM.

Design, synthesis，biological evaluation and docking studies of novel 2-substituted-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives as dual PI3Kα/mTOR inhibitors

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Four series of 2-substituted-4-morpholino- 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives (**9**–**28**) were designed, synthesized and their structures were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectrum . All compounds were evaluated for the IC_{50} values against three cancer cell lines (A549, PC-3 and MCF-7). And four selected compounds (**10**, **11**, **24**, **27**) were further evaluated for the IC_{50} values against PI3Kα and mTOR kinases. Seven of the target compounds exhibited moderate to excellent antitumor activities against these three cancer cell lines. The most promising compound **11** showed good antitumor potency for A549, PC-3 and MCF-7 cell lines with IC_{50} values of 0.52 ± 0.10 μM, 1.41 ± 0.10 μM, 4.82 ± 0.24μM and moderate antitumor activities against PI3Kα/mTOR with IC_{50} values of 6.72 ± 0.30 μM and 0.94 ± 0.10 μM. Structure–activity relationships (SARs) and docking studies indicated that aryl urea scaffolds had a significant impact on the antitumor activities, and aryl pyridine urea scaffolds produced the best potency. Variations in substitutions of the aryl group had a significant impact on the activity and 3-Cl-4-F or 3-CF_3-4-Cl substitution was more preferred.

Key words: Thiopyrano[4,3-*d*]pyrimidine; Synthesis; Docking; PI3Kα/mTOR inhibitors

1. Introduction

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The phosphatidylinositol 3-kinase (PI3K) / protein kinase B (Akt) / the mammalian target of rapamycin (mTOR) pathway is an intracellular signaling pathway important in regulating various cellular processes, including growth regulation, apoptosis, and survival in many cancers [1-3]. In recent years, many (fused-)pyrimidine/triazine derivatives were reported as PI3K-Akt-mTOR signal pathway inhibitors (Figure 1) [4-9]. Among them, GDC-0941, which is a potent, orally bioavailable inhibitor of PI3K, exerted antitumor activity against an array of human tumor cell lines and is currently undergoing Phase II clinical trials [10]. PF-05139962 [6], compound [7], PKI-402[11] and PKI-587[12] (Figure 1) are several aryl urea derivatives which exhibit potent anti-tumor activity. Structure-activity relationships (SARs) showed that aryl urea moieties were essential for the anti-tumor activity.

In our previous research, we reported several series of analogous of PF-05139962, 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine hydrazone (Figure 1) which demonstrated excellent antitumor activity. Biological evaluation results showed that thiopyrano[4,3-*d*]pyrimidines were important for the activity of the target compounds.

In continuation of our previous research, and in order to screen compounds which possess excellent *in vitro* / *in vivo* anti-tumor activity as well as improved pharmacokinetic properties, further studies on analogous of PF-05139962 were carried out in this research. The design strategy for all target compounds is shown in Figure 2 and is described as follow. Firstly, the urea group was kept unchanged, and a series of phenyl pyridine urea compounds **9**–**12** was designed by replacing the ethyl group of PF-05139962 with aryl group and replacing the phenyl group with pyridine group. What's more, sulfur atom was oxidized to study the influence to the target compounds. This series of compounds showed excellent cytotoxicity and moderate PI3Kα/mTOR kinases inhibitory activity. Furthermore, investigations were carried out in details to study the effect of urea to the activity and therefore urea was replaced by sulfonylurea group to yield compounds **13**–**18** and **19**–**22**. However, this two series of compounds showed poor cytotoxicity and moderate mTOR kinase inhibitory activity. Thus, we shifted our focus back to the phenyl pyridine urea series. To investigate the influences of pyridine urea to the activity and inspired by previous compounds 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine hydrazone (Compound Ⅱ in Figure 2), phenyl pyridine urea was replaced by piperazine phenylurea to afford compounds **23**–**28**. The activity of **23**–**28** was similar to that of **9**–**12** and was much better than that of compounds **13**–**18** and **19**–**22**.

The studies of Wentao Yue showed that PI3K, Akt and mTOR gene of the PI3K-Akt-mTOR signaling pathway expression in the non-small cell lung cancer were (61±23)%, (77±32)%, (43±21)%

respectively[13].And the level of this pathway in breast cancer also up to 70%[14]. In the prostate cancer cells, the missing of tumor suppressor gene (PTEN) resulted in the high expression of PI3K-Akt-mTOR signaling pathway. In addition, the studies of GDC-0941 showed it has strong inhibitory activities against A549(lung cancer cell lines), PC-3 (prostate cancer cell lines)and MCF-7(breast cancer cell lines) , suggesting that these three cells have a high sensitivity for PI3K-Akt-mTOR signaling pathway inhibitors[15-17].Therefore, A549,PC-3 and MCF-7 cell lines were chose as the test cell line for in vitro antitumor experiments in this study.

Herein we disclosed the design, synthesis and antitumor activity of four series of compounds against A549, PC-3, MCF-7 and PI3Kα, mTOR and c-Met kinases. Moreover, docking studies were presented in this paper as well.

(**Fig. 1.** should be listed here.)

(**Fig. 2.** should be listed here.)

2. Chemistry

The preparation of target compounds **9**–**28** was described in Schemes 1 and 2.

Compounds **7a**–**d** and **8a**–**e** were synthesized according to the procedures reported previously by our group [18-19]. The different substituent of phenylamine reacted with triphosgene to get compound compounds **7a**–**d**. **8a**–**e** was synthesized from the different substituent of chlorobenzene through three steps.

The key intermediates 4-(2-chloro-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (**4a**) and 2-chloro-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide(**4b**) were synthesized according to the procedures reported previously by our group [5].

Compounds **5a** and **5b** were synthesized according to the following procedure. **4a** was reacted with 5-bromopyridin-2-amine through Suzuki-coupling reaction using bis(triphenylphosphine)palladium(II) dichloride as catalyst to generate compound **5a**. Similarly, Compound **5b** was synthesized from **4b** through Suzuki-coupling reaction. And compound **4a** was reacted with 1-bromo-4-nitrobenzene through Suzuki-coupling reaction and then reduction by $NH_2NH_2{\cdot}H_2O$ (80%) yielding compound 4-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)aniline **5c**. At the same way, 2-(4-aminophenyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide **5d** could be synthesized from compound **4b** through the same methods.

Substitution of **4a**–**b** with piperazine achieved **6a**–**b**.

Finally, key intermediates **5a**–**d** and **6a**–**b** condensed with **7a**–**d** or **8a**–**e** to afford target compounds **9**–**28**, respectively.

(**Scheme 1.** should be listed here.)

(**Scheme 2.** should be listed here.)

3. Results and discussion

3.1 Biological evaluation

The target compounds (**9**–**28**) were evaluated for the cytotoxicity against three cancer cell lines A549, PC-3 and MCF-7 by 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cell proliferation assay, together with reference compounds sorafenib and GDC-0941. In addition, four selected compounds **10**, **11**, **24** and **27** were further evaluated for their IC_{50} values against mTOR, PI3Kα, c-Met kinases by LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay, Kinase-Glo® Luminescent Kinase Assay or by Mobility shift assay against c-Met with ATP concentration at Km. The results expressed as IC_{50} values are summarized in Tables 1 and 2 and the values are the average of at least two independent experiments.

(**Table 1.** should be listed here.)

As illustrated in Table 1, seven of the synthesized compounds showed moderate to significant cytotoxic activities with IC_{50} valuables in single-digit μM to nanomole range and four of them (**10**, **11**, **24**, **27**) showed excellent cytotoxic activity with IC_{50} values from 6.13 ± 0.62 μM to 0.52 ± 0.10 μM which were equal to more potent than sorafenib and GDC-0941.

Furthermore, we can easily find that the phenyl pyridine urea compounds **9**–**12** showed excellent cytotoxic activity and but sulfonylurea derivatives **13**–**22** showed no activity. It promoted us that replacing the ethyl group of PF-05139962 with aryl groups and replacing the phenyl urea scaffold with pyridine urea scaffold didn't decrease the activity. However, when the urea group was replaced by sulfonylurea, the target compounds lost biological activity. When the phenyl pyridine urea was replaced by piperazine phenyl urea, the activity of **23**–**28** were similar to that of **9**–**12** and were much better than that of compounds **13**–**22**.The results suggested that

aryl urea scaffold played an important role in the antitumor activity of target compounds and the pyridine group was not essential for the target compounds. No matter the sulfur atom was oxidized or not, the activity wasn't changed remarkablely. What's more, variations in substitutions of the aryl group had a significant impact on the activity, and for the piperazine phenyl urea and phenyl pyridine urea series, 3-Cl-4-F (**10**–**11**) or 3-CF_3-4-Cl (**12**, **24**, **27**) substitution were more preferred.

The antitumor activities of the four selected compounds (**10**, **11**, **24**, **27**) as well as the lead compound GDC-0941 against PI3Kα, mTOR and c-Met kinases were shown in Table 2. All the four selected compounds showed well antitumor activity toward PI3Kα, mTOR kinases, especially compounds **10** and **11** with IC_{50} values in nanomole range. However, the activity against c-Met kinase is bad. This result told us that these compounds showed high selectivity toward PI3Kα/mTOR to c-Met kinases. What's more, the excellent kinases activity of **10**, **11**, **24**, **27** prompt us that linker urea group is essential for the target compounds and piperazine urea or pyridylurea group were more preferred.

(**Table 2.** should be listed here.)

3.2 Molecular docking study

To explore the binding modes of target compounds with the active site of mTOR and PI3Kα, molecular docking simulation studies were carried out by using SURFLEX-DOCK module of SYBYL package version. Based on the *in vitro* inhibition results, we selected four representative compounds **10**, **11**, **24** and **27**, our best mTOR/PI3Kα inhibitor in this study, as ligand examples, and the structures of mTOR (PDB ID code:4JT6 [20]) and PI3Kα (PDB ID code:4L23 [21]) were selected as the docking models. In this study, we chose compound **11** to explaining the docking model.

(**Fig. 3.** should be listed here.)

The binding modes of compound **11** and lead compound were shown in Fig. **3a**-**b**. The docking scores were shown in Table 2. As depicted in Fig. **3a**, compound **11** and PI103 can overlap in the position of morpholine group in the binding model and the pyridine and urea group of bis-aryl urea moiety formed four hydrogen bonds with residues ASP2195 and ASP2357 in the docking model of mTOR, respectively.The morpholine group formed one hydrogen bond with residues VAL2240.Similarly, the hydrogen bonds were found in the docking model of PI3Kα in Fig. **3b**, such as the hydrogen bonds between morpholine group and residue VAL851, the bis-aryl urea moiety and the residues LYS802 and ASP810 and the double oxide thiopyran moiety can also

formed one hydrogen bonds with residue GLN859. The activity against mTOR/PI3Kα kinases of compound **11** was different, which was attributed to the difference of the hydrogen bonds between the bis-aryl urea moiety and amino acid residues. Analysis of compound **11**'s binding mode in the active binding site and the results of activity suggested that the several hydrogen bonds, formed in morpholine group and the bis-aryl urea moiety, really plays an important role in increasing the inhibitory potency of 2-substituted-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives against mTOR/PI3Kα kinases. The docking results indicated that these compounds have plausible binding modes to both enzymes.

The molecular docking scores in Table 2 show that docking score of pyridine aryl urea compounds is better than that of pyridine sulfonyl urea compounds and benzene sulfonyl urea compounds. The docking score of piperizine aryl urea compounds is the lowest**.** Generally, the docking results were agreed with the biological evaluation results of target compounds.

Furthermore, the docking results also give us a new direction to design new mTOR/PI3Kα inhibitors that can interact with ASP2195, ASP2357, LYS802, ASP810 and GLN859. The above-mentioned results of SAR analysis and molecular docking study may allow the rational design of more potent mTOR/Pi3kα inhibitors.

Conclusions

In summary, four series of 4-(2-chloro-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine derivatives were designed, synthesized and evaluated for antitumor activity against three cancer cell lines and mTOR, PI3Kα and c-Met kinases *in vitro.* The pharmacological results indicated that seven of the synthesized compounds displayed moderate antitumor activity and four selected compounds showed equal to more potency than lead compound sorafenib and GDC-0941. The most promising compound **11** exhibited strong antitumor activities against A549, PC-3 and MCF-7 cell lines, with IC_{50} values of 0.52 ± 0.10 μM, 1.41 ± 0.10 μM,4.82±0.24μM and moderate antitumor activities against PI3Kα/mTOR with IC_{50} values of 6.72 ± 0.30 μM and 0.94 ± 0.10 μM. The initial SARs and docking studies showed that aryl urea moieties were necessary for the activity of these compounds and aryl pyridine urea scaffolds produced the best potency. Variations in substitutions of the aryl urea moieties had a significant impact on the activity and 3-Cl-4-F or 3-CF_3-4-Cl substitution was more preferred. Further study will be carried out in near future.

5. Experimental

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. NMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LCMS (Agilent, Palo Alto, CA, USA). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specied. Yields were not optimized.

*5.2. Preparation of compounds **5a**–**d** and **6a**–**b***

Compounds **5a**–**d** and **6a**–**b** were synthesized from compound **1** according to the procedures in our previous research [5].

*5.3. General procedure for Compounds **7a**–**d** and **8a**–**e***

Compounds ***7a***–***d*** *and* ***8a***–***e*** were synthesized according to the procedures in our previous research [18-19] .

*5.3 General procedure for the preparation of compounds **9**–**12***

A mixture of 5-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)pyridin-2-amine **5a** (0.10g, 0.30mmol) or 2-(6-aminopyridin-3-yl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide **5b** (0.10g, 0.28mmol), substitued isocyanatobenzene **7a**–**d** in 1,4-dioxane(15mL) was refluxed for 10-30 mins. The mixture was washed with H_2O and separated by filtration to afford white solids **9**–**12.**

*5.3.1 1-(5-(4-Morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)-3-phenylurea (**9**)*

This compound was obtained as white solid in 85.2% yield. m.p.: 261.2–262.3 □; ESI-MS m/z: 449.2$[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 10.46 (s, 1H, NH), 9.68 (s, 1H, NH), 9.19 (s, 1H, NCH), 8.55 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.62 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.56 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.32 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.32-7.02 (m, 1H, Ar-H), 3.82–3.76 (m, 6H, OCH_2/SCH_2), 3.40 (s, 4H, NCH_2), 3.07 (d, *J* = 5.2 Hz, 2H, SCH_2), 3.01 (d, *J* = 5.5 Hz, 2H, CH_2).

*5.3.2 1-(3-Chloro-4-fluorophenyl)-3-(5-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)urea (**10**)*

This compound was obtained as white solid in 87.2% yield. m.p.: 245.2–247.1 □; ESI-MS m/z: 535.1$[M+Cl]^-$; ^{1}H NMR (400 MHz, DMSO) *δ* 10.88 (s, 1H, NH), 9.82 (s, 1H, NH), 9.20 (s, 1H, NCH), 8.55 (d, *J* = 8.6 Hz, 1H,

Ar-H), 7.93 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.56 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.51 – 7.46 (m, 1H, Ar-H), 7.36 (t, *J* = 9.0 Hz, 1H, Ar-H), 3.80–3.74 (m, 6H, OCH_2/SCH_2), 3.39 (s, 4H, NCH_2), 3.06 (d, *J* = 5.6 Hz, 2H, CH_2), 3.01 (d, *J* = 5.3 Hz, 2H, SCH_2).

5.3.3

*1-(3-Chloro-4-fluorophenyl)-3-(5-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)urea (**11**)*

This compound was obtained as white solid in 86.1% yield. m.p.: 285.2–286.1 □; ESI-MS m/z: 533.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 10.82 (s, 1H), 9.85 (s, 1H, NH), 9.22 (s, 1H, NH), 8.57 (d, *J* = 8.6 Hz, 1H, NCH), 7.93 (d, *J* = 4.5 Hz, 1H, Ar-H), 7.58 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.48 (s, 1H, Ar-H), 7.37 (t, *J* = 9.0 Hz, 1H, Ar-H), 4.37 (s, 2H, SCH_2), 3.77 (s, 4H, OCH_2), 3.57 (s, 2H, SCH_2), 3.39 (s, 6H, NCH_2/CH_2).

5.3.4

*1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(5-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)urea (**12**)*

This compound was obtained as white solid in 89.1% yield. m.p.: 223.1–224.2□; ESI-MS m/z: 583.0$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 11.01 (s, 1H, NH), 10.01 (s, 1H, NH), 9.22 (s, 1H, NCH), 8.60 (d, *J* = 8.7 Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.78 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.78 –7.68 (m, 2H, Ar-H), 4.39 (s, 2H, SCH_2), 3.77 (s, 4H, OCH_2), 3.57 (s, 2H, SCH_2), 3.47-3.43 (m, 6H, NCH_2/CH_2).

*5.4. General procedure for the preparation of compounds **13**–**22***

A mixture of **5a** (0.10g, 0.30mmol), or **5b** (0.10g, 0.27 mmol), or 4-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)aniline **5a** (0.10g, 0.30mmol), or **5b**(0.10g, 0.28mmol), substitued sulfonylurea **8a**–**e** in methylbenzene(10 mL) was refluxed for 4-8h. The mixture was separated by filtration to afford the solids **13**–**22**

5.4.1

*N-((5-(4-Morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carbamoyl)thiophene-2-sulfonamide (**13**)*

This compound was obtained as white solid in 66.2% yield. m.p.: 226.4–227.5 □; ESI-MS m/z: 519.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.66 (s, 1H, NH), 8.83 (s, 1H, Ar-H), 7.83 (d, *J* = 4.0 Hz, 1H, Ar-H), 7.55 (s, 3H, Ar-H), 7.14 (d, *J* = 2.7 Hz, 1H, Ar-H), 6.72 (s, 1H, NH), 3.83 – 3.63 (m, 8H, NCH_2/OCH_2), 3.10 – 2.93 (m, 6H, SCH_2/CH_2).

5.4.2

*4-Chloro-N-((5-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carbamoyl)benzenes ulfonamide (**14**)*

This compound was obtained as white solid in 75.3% yield. m.p.: 216.1–217.5 □; ESI-MS m/z: 548.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.70 (s, 1H, NH), 8.83 (s, 1H, Ar-H), 7.82 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.65 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.60 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.37 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.75 (s, 1H, NH), 3.80 – 3.64 (m, 8H, NCH_2/OCH_2), 3.11 – 2.95 (m, 6H, SCH_2/CH_2).

5.4.3

*4-Fluoro-N-((5-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carbamoyl)benzenes ulfonamide (**15**)*

This compound was obtained as white solid in 80.1% yield. m.p.: 216.8–218.2□; ESI-MS m/z: 531.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.69 (s, 1H, NH), 8.85 (s, 1H, Ar-H), 7.88 (dd, *J* = 7.8, 5.8 Hz, 2H, Ar-H), 7.52 –7.45 (m, 1H, Ar-H), 7.43 –7.35 (m, 2H, Ar-H), 7.15 (dd, *J* = 15.6, 7.1 Hz, 1H, Ar-H), 6.74 – 6.44 (m, 1H, NH), 3.84 – 3.63 (m, 8H, NCH_2/OCH_2), 3.10 – 2.89 (m, 6H, SCH_2/CH_2).

5.4.4

*2,4-Difluoro-N-((5-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carba moyl)benzenesulfonamide (**16**)*

This compound was obtained as white solid in 60.1% yield. m.p.: 190.4–191.2 □; ESI-MS m/z: 581.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.66 (s, 1H, NH), 8.86 (s, 1H, Ar-H), 7.86 (dd, *J* = 15.2, 8.1 Hz, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 7.10 (s, 2H, Ar-H), 6.80 (s, 1H, NH), 4.40–4.34 (m, 2H, SCH_2), 3.75 (s, 4H, OCH_2), 3.54 (d, *J* = 5.7 Hz, 2H, SCH_2), 3.38 (d, *J* = 5.0 Hz, 6H, NCH_2/CH_2).

5.4.5

*4-Methyl-N-((5-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carbamo yl)benzenesulfonamide (**17**)*

This compound was obtained as white solid in 65.3% yield. m.p.: 202.8–203.2 □; ESI-MS m/z: 559.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.69 (s, 1H, NH), 8.88 (s, 1H, Ar-H), 7.87 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.71 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.36 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.17 (d, *J* = 7.3 Hz, 1H, Ar-H), 6.56 (s, 1H, NH), 4.34 (d, *J* = 17.8 Hz, 2H, SCH_2), 3.75 (s, 4H, OCH_2), 3.54 (d, *J* = 6.4 Hz, 2H, SCH_2),3.45–3.39 (m, 6H, NCH_2/CH_2), 2.38 (d, *J* = 9.2 Hz, 3H, CH_3).

5.4.6

*4-Chloro-N-((5-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)pyridin-2-yl)carbamoyl)benzenesulfonamide (**18**)*

This compound was obtained as white solid in 75.2% yield. m.p.: 203.8–204.4 □; ESI-MS m/z: 579.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.76 (s, 1H, NH), 8.86 (s, 1H, Ar-H), 7.96 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.83 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.37 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.78 (s, 1H, NH), 4.40–4.34 (m, 2H, SCH_2), 3.75 (s, 4H, OCH_2), 3.55 (d, *J* = 6.1 Hz, 2H, SCH_2), 3.46–3.38 (m, 6H, NCH_2/CH_2).

5.4.7

*4-Chloro-N-((4-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)carbamoyl)Benzenesulfonamide (**19**)*

This compound was obtained as white solid in 79.2% yield. m.p.: 214.5–216.2 □; ESI-MS m/z: 547.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.24 (s, 1H, NH), 8.31 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.12 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.92 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.79 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.69 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.54 (s, 2H, Ar-H), 7.46 (d, *J* = 7.9 Hz, 1H, Ar-H), 6.70 (d, *J* = 8.0 Hz, 1H, NH), 3.84 (s, 4H, OCH_2), 3.78 (d, *J* = 6.1 Hz, 2H, SCH_2), 3.45 (s, 4H, NCH_2), 3.20 – 3.05 (m, 4H, SCH_2/CH_2).

*5.4.8 4-Fluoro-N-((4-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)carbamoyl)benzenesulfonamide (**20**)*

This compound was obtained as white solid in 70.2% yield. m.p.: 220.1–222.3 □; ESI-MS m/z: 530.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.12 (s, 1H, NH), 8.31 (d, *J* = 8.5 Hz, 1H, Ar-H), 8.12 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.95 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.80 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.53 (d, *J* = 4.8 Hz, 2H, Ar-H), 7.45 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 6.69 (d, *J* = 8.4 Hz, 1H, NH), 3.84 (s, 4H, OCH_2), 3.78 (d, *J* = 8.3 Hz, 2H, SCH_2), 3.43 (s, 4H, NCH_2), 3.08 (d, *J* = 5.3 Hz, 4H, SCH_2/CH_2).

5.4.9

*4-Chloro-N-((4-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)carbamoyl)benzenesulfonamide (**21**)*

This compound was obtained as white solid in 65.1% yield. m.p.: 232.4–233.1 □; ESI-MS m/z: 578.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.33 (s, 1H, NH), 8.33 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.14 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.92 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.81 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.69 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.62–7.56 (m,

2H,Ar-H), 7.46 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.71 (d, *J* = 7.9 Hz, 1H, NH), 4.41–4.34 (m, 2H, SCH_2), 3.85 (s, 4H, OCH_2), 3.63 (d, *J* = 6.0 Hz, 2H, SCH_2), 3.44 (s, 6H, NCH_2/CH_2).

5.4.10

*4-Chloro-N-((4-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)carbamoyl)benzenesulfonamide (**22**)*

This compound was obtained as white solid in 76.1% yield. m.p.: 254.1–255.2□; ESI-MS m/z: 562.1$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.23 (s, 1H, NH), 8.31 (d, *J* = 16.0 Hz, 2H, Ar-H), 8.14 (d, *J* = 8.2 Hz, 2H), 7.60–7.54 (m, 4H, Ar-H), 6.70 (d, *J* = 8.2 Hz, 1H, NH),4.50–4.41 (m, 2H, SCH_2), 3.84 (s, 4H, OCH_2), 3.63 (d, *J* = 6.4 Hz, 2H, SCH_2), 3.43 (s, 6H, NCH_2/CH_2)

*5.5 General procedure for the preparation of compounds **23**–**28***

A mixture of 4-(2-(piperazin-1-yl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine **6a** (0.10g, 0.31mmol) or 4-morpholino-2-(piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine 6,6-dioxide **6b** (0.10g, 0.28mmol), substituted isocyanatobenzene **7a**–**c** in 1,4-dioxane(10mL) was refluxed for 10-30 min. The mixture was washed with 10mL H_2O and separated by filtration to afford the solids **23**–**28**

*5.5.1 4-(4-Morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)-N-(p-tolyl)piperazine-1-Carboxamide (**23**)*

This compound was obtained as white solid in 85.2% yield. m.p.: 233.1–234.4 □; ESI-MS m/z: 441.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 8.57 (s, 1H,NH), 7.47 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.8 Hz, 2H, Ar-H), 6.94 (t, *J* = 7.3 Hz, 1H,Ar-H), 3.71 (d, *J* = 3.9 Hz, 8H, NCH_2), 3.57 (d, *J* = 5.4 Hz, 2H,CH_2), 3.51 (d, *J* = 5.0 Hz, 4H, CH_2), 3.22 (s, 4H,OCH_2), 2.88 (dd, *J* = 16.6, 5.4 Hz, 4H, NCH_2); ^{13}C NMR (101 MHz, DMSO) *δ* 165.47(C), 165.18(C), 159.17(C), 155.58(C), 140.96(C), 128.77(2CH), 122.24(C), 120.15(2CH), 107.34(CH), 66.43($2CH_2$), 49.36($2CH_2$), 44.02($2CH_2$), 43.83($2CH_2$), 34.01(CH_2), 26.19(CH_2), 25.93(CH_2).

5.5.2

*N-(4-Chloro-3-(trifluoromethyl)phenyl)-4-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazine-1-carboxamide (**24**)*

This compound was obtained as white solid in 88.1% yield. m.p.: 218.2–220.4 □; ESI-MS m/z: 543.2$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) *δ* 9.47 (s, 1H,NH), 8.17 (s, 1H,Ar-H), 7.90 –7.82 (m, 1H, Ar-H), 7.56 (d, *J* = 8.8 Hz, 1H, Ar-H), 3.87 (s, 4H,OCH_2), 3.69 (s, 8H, NCH_2), 3.67 (s, 2H, SCH_2), 3.64 (s, 4H,NCH_2), 3.12 (t, *J* = 5.2 Hz,

2H, CH_2), 2.93 (t, *J* = 5.9 Hz, 2H, SCH_2); ^{13}C NMR (101 MHz, DMSO) *δ* 163.26(C), 154.95(C), 140.78(C), 131.97(C), 126.90(C), 126.60(C), 124.76(C), 124.42(C), 122.59(CH), 122.05(C), 118.44(CH), 106.81(CH), 66.41($2CH_2$),48.80($2CH_2$), 45.23($2CH_2$), 43.59($2CH_2$), 29.96(CH_2), 26.30(CH_2), 24.74(CH_2).

*5.5.3 N-(4-Bromophenyl)-4-(4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazine-1-Carboxamide (**25**)*

This compound was obtained as white solid in 88.9% yield. m.p.: 209.5–212.2 □; ESI-MS m/z: 521.1$[M+H]^+$; ^1H NMR (400 MHz, DMSO) *δ* 8.84 (s, 1H, NH), 7.49 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.40 (d, *J* = 8.2 Hz, 2H, Ar-H), 3.74 (s, 6H, $2NCH_2/SCH_2$), 3.69 (s, 4H, OCH_2), 3.64 – 3.47 (m, 8H, NCH_2), 2.91 (s, 4H,SCH_2/ CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.44(C), 155.25(C), 140.48(C), 140.48(C), 136.97(C), 131.52(2CH), 121.92(2CH), 113.73(C), 107.16(C), 66.43($2CH_2$), 49.16($2CH_2$), 44.26($2CH_2$), 43.84($2CH_2$), 26.09($2CH_2$), 25.76(CH_2).

5.5.4

*4-(4-Morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (**26**)*

This compound was obtained as white solid in 83.1% yield. m.p.: 248.2–249.9 □; ESI-MS m/z: 495.2$[M+Na]^+$; ^1H NMR (400 MHz, DMSO) *δ* 8.59 (s, 1H, NH), 7.47 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.8 Hz, 2H, Ar-H), 6.94 (t, *J* = 7.3 Hz, 1H, Ar-H), 4.16 (s, 2H, SCH_2), 3.72 (d, *J* = 3.7 Hz, 8H, NCH_2), 3.51 (s, 4H, OCH_2), 3.44 (t, *J* = 6.5 Hz, 2H, SCH_2), 3.20 (s, 4H, NCH_2), 3.15 (t, *J* = 6.7 Hz, 2H, CH_2)

5.5.5

*N-(4-Chloro-3-(trifluoromethyl)phenyl)-4-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazine-1-carboxamide (**27**)*

This compound was obtained as white solid in 89.1% yield. m.p.: 254.1–256.3 □; ESI-MS m/z: 597.1$[M+Na]^+$; ^1H NMR (400 MHz, DMSO) *δ* 9.19 (s, 1H, NH), 8.10 (s, 1H, Ar-H), 7.83 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.14 (s, 2H, SCH_2), 3.80–3.71 (m, 8H, NCH_2), 3.55 – 3.50 (m, 4H, OCH_2), 3.42 (d, *J* = 6.3 Hz, 2H,SCH_2), 3.18 (s, 4H, NCH_2), 3.13 (t, *J* = 6.6 Hz, 2H, CH_2).

5.5.6

*N-(4-Bromophenyl)-4-(4-morpholino-6,6-dioxido-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazine-1-carboxamide (**28**)*

This compound was obtained as white solid in 90.3% yield. m.p.: 264.1–265.5 □; ESI-MS *m/z*: 573.1$[M+Na]^+$; ^1H NMR (400 MHz, DMSO) *δ* 8.73 (s, 1H, NH), 7.45 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.39 (d, *J* = 7.9 Hz, 2H,Ar-H),

4.14 (s, 2H, SCH_2), 3.70 (s, 8H, NCH_2), 3.50 (s, 4H, OCH_2), 3.42 (s, 2H, SCH_2), 3.18 (s, 4H, NCH_2), 3.14 (d, *J* = 6.1 Hz, 2H, CH_2).

5.6 *Cytotoxicity assay in vitro*

The cytotoxicity of target compounds (**9**–**28**) were evaluated with A549, PC-3, MCF-7 cell lines by the standard MTT assay *in vitro*, with Sorafenib and GDC-0941 as positive controls. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 ℃ for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 mg/mL and incubated with cells at 37℃ for 4 h. T he formazan crystals were dissolved in 100 mL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the referencewavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as inhibition rates or IC_{50} (half-maximal inhibitory concentration) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

5.7 *mTOR Kinase Assay*

The mTOR kinase activity of four selected compounds (**10**, **11**, **24**, **27**) were determined using LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay following the manufacturer's instructions, with GDC-0941 and PI103 as positive controls [22]. Briefly, mTOR enzyme (10 nM), ATP (21.6 μM), ULight-4E-BP1 Peptide (100 nM) and test compounds were diluted in kinase buffer (50 mM HEPES pH 7.5.1 mM EGTA, 3 mM MnCl2, 10 mM $MgCl_2$, 2 mM DTT and 0.01%Tween-20). The reactions were performed in white 384-well Optiplates (PerkinElmer, MA, USA) at room temperature for 1 h and stopped by adding EDTA to 16 mM. Eu-antiphospho-4E-BP1 (Thr37/46) Antibody (PerkinElmer, MA, USA) was then added to each well to a final concentration of 2 nM. The intensity of the light emission was measured with an EnVision® Multilabel Reader (PerkinElmer, MA, USA) in TR-FRET mode (excitation at 320 nm and emission at 665 nm). All of the compounds were tested two times. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations.

5.8 PI3Kα kinase assay

Four selected compounds (**10**, **11**, **24**, **27**) are tested for their activity against PI3K*α* using a Kinase-Glo® Luminescent Kinase Assay, with GDC-0941 and PI103 as positive controls[23]. The kinase reaction is done in 384-well black plate. Each well is loaded with 50 mL of test items (in 90% DMSO) and 5 mL reaction buffer containing 10 mg/mL PI substrate (L-a-phosphatidylinositol; Avanti Polar Lipids; prepared in 3% octyl-glucoside) and the PI3kα protein 10 nM is then added to it. The reaction is started by the addition of 5 mL of 1 mMATP prepared in the reaction buffer and is incubated for 60 min for p110α.It is terminated by the addition of 10 mL Kinase-Glo buffer. The plates are then read in a Synergy 2 reader for luminescence detection. All of the compounds were tested two times. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations.

5.9 c-Met kinase assay

Four selected compounds (**10**, **11**, **24**, **27**) are tested for their activity against c-Met kinase through the mobility shift assay [24]. All kinase assays were performed in 96-well plates in a 50 μL reaction volume. The kinase buffer contains 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 0.0015% Brij-35 and 2 mM DTT. The stop buffer contains 100 mM HEPES, pH 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3 and 50 mM EDTA. Dilute the compounds to 500 μM by 100% DMSO, then transfer 10 μL of compound to a new 96-well plate as the intermediate plate, add 90 μL kinase buffer to each well. Transfer 5 μL of each well of the intermediate plate to 384-well plates. The following amounts of enzyme and substrate were used per well: kinase base buffer, FAM-labeled peptide, ATP and enzyme solution. Wells containing the substrate, enzyme, DMSO without compound were used as DMSO control. Wells containing just the substrate without enzyme were used as low control. Incubate at room temperature for 10 min. Add 10 μL peptide solution to each well. Incubate at 28 ℃ for specified period of time and stop reaction by 25 μL stop buffer. At last collect data on Caliper program and convert conversion values to inhibition values. Percent inhibition = (max − conversion)/(max − min) × 100. “max” stands for DMSO control; “min” stands for low control.

5.10 *Docking studies*

For docking purposes, we prepared the receptor proteins PDB ID code:4JT6(mTOR) and and PDB ID code:4L23(PI3Kα). The three-dimensional structures of the mTOR (PDB code: 4JT6) and PI3Kα (PDB ID code:4L23) [20-21] were obtained from RCSB Protein Data Bank. We built a small organic molecules set

(compound **10**, **11**, **24**, **27**) and used Gasteiger-Huckel to optimized the molecular force field and structure. Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. First of all, extract ligand substructure, then remove water and excess structure, finally, add hydrogens and fix sidechain amides. The protonated state of several important residues were adjusted by using SYBYL6.9.1 (Tripos, St. Louis, USA) in favor of forming reasonable hydrogen bond with the ligand. Molecular docking analysis was carried out by the SURFLEX-DOCK module of SYBYL 6.9.1 package to explore the binding model for the active site of mTOR or PI3Kα with its ligand. All atoms located within the range of 5.0 Å from any atom of the cofactor were selected into the active site, and the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was selected. Other default parameters were adopted in the SURFLEX-DOCK calculations. All calculations were performed on Silicon Graphics workstation. Lastly, docking resultsand the optimized molecular docking model with the receptor proteins was obtained.

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Legends

Fig. 1 Structures of some reported (fused-)pyrimidine/triazines

Fig. 2 Structures and design strategy for target compounds **9**–**28**

Fig. 3a-b. Binding models of compound **11** and native ligand PI 103 target into active site of mTOR(Fig.3a) and PI3Kα(Fig.3b). The proteins were displayed by cyan and white ribbon. Compound **11** and lead compound were displayed by orange and green sticks, respectively. H-bonding interactions between the **11**, lead compound and mTOR/PI3Kα were indicated with dashed lines in yellow.

Scheme 1. Synthetic routes of key intermediates **5a**–**d** and **6a**–**b**

Reagents and conditions: (a) NaH, THF, rt, 3 h; (b) 3 equiv urea, C_2H_5ONa, EtOH, reflux, 24 h; (c) $POCl_3$, DMF(cat.), reflux, 3 h; (d) 2.4 equiv morpholine, MeOH, rt, 1.5 h; (e) $Na_2WO_4 \cdot 2H_2O$, 30% H_2O_2, 20℃, 3 h; (f) 1)bis(pinacolato)diboron, KAc, $Pd(PPh_3)_2Cl_2$, 1,2-dimethoxyethane, reflux, 2 h; 2) H_2O, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, reflux, 6 h; (g) $NH_2NH_2 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$, actived C, EtOH, 1 h; (h) EtOH, K_2CO_3, reflux, 2–3 h;

Scheme 2. Synthetic routes of target compounds **9**–**12**, **13**–**18**, **19**–**22** and **23**–**28**

Reagents and conditions: (a) 1,4-dioxane, H_2O, rt, 10–30mins; (b) methylbenzene, rt, 4–8h.

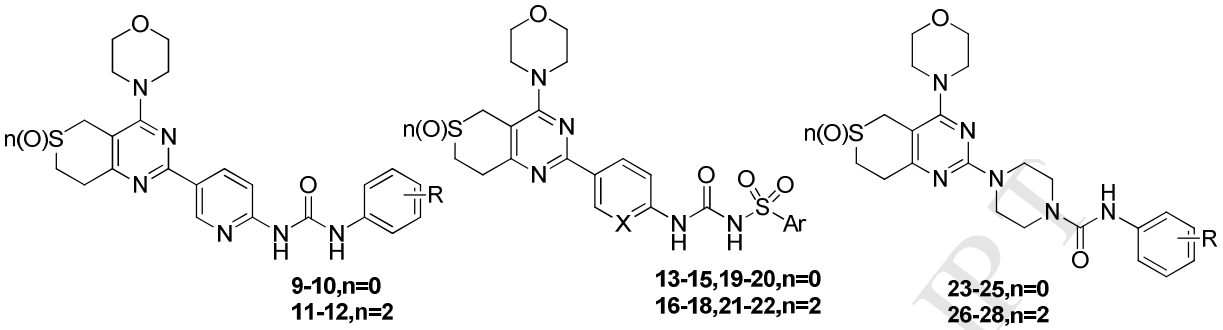
Table 1 Structures and cytotoxicity of target compounds **9**–**28**

[a] The values are an average of two separate determinations.
[b] Used as a positive control
[c] NA: Not active (IC_{50} > 50 μM)
[d]ND: Not determined

Table 2 Melocular docking scores and mTOR/PI3Kα kinases activity of selected compounds and positive controls.

[a] The values are an average of two separate determinations.
[b] Used as a positive control
[c]ND: Not determined

Table 1 Structures and cytotoxicity of target compounds **9**–**28**



Comp. No.	R(**9**–**12**,**23**–**28**) /Ar(**13**–**22**)	IC_{50}[a] (μM)		
		A549	PC-3	MCF-7
9	H	**1.27±0.32**	NA[c]	46.62±1.12
10	3-Cl-4-F	**6.07±0.61**	20.52±0.92	11.47±0.76
11	3-Cl-4-F	**0.52±0.10**	**1.41±0.10**	**4.82±0.24**
12	3-CF_3-4-Cl	NA	32.24±0.96	**4.67±0.42**
13	thiophene	46.88±1.05	NA	NA
14	4-Cl-phenyl	NA	NA	NA
15	4-F-phenyl	36.17±0.98	NA	NA
16	2,4-diF-phenyl	22.67±0.89	NA	19.65±0.88
17	4-CH_3- phenyl	42.46±1.02	NA	NA
18	4-Cl-phenyl	NA	NA	NA
19	4-Cl-phenyl	NA	NA	NA
20	4-F-phenyl	NA	NA	NA
21	4-Cl-phenyl	NA	NA	NA
22	4-F-phenyl	NA	NA	NA
23	H	NA	NA	NA
24	3-CF_3-4-Cl	**3.83±0.32**	26.73±0.85	**5.80±0.52**
25	4-Br	39.90±0.89	NA	**4.68±0.41**
26	H	NA	NA	NA
27	3-CF_3-4-Cl	**6.13±0.62**	28.73±0.80	**5.94±0.50**
28	4-Br	19.01±0.87	NA	45.57±0.95

Sorafenib[b]	-	6.53±0.82	8.08±0.91	4.21±0.62
GDC-0941[b]	-	1.2 ± 0.11	0.8 ± 0.44	ND[d]

[a] The values are an average of two separate determinations.

[b] Used as a positive control

[c] NA: Not active (IC_{50} > 50 μM)

[d]ND: Not determined

Table 2 Melocular docking scores and mTOR/PI3Kα kinases activity of selected compounds and positive controls.

Compound NO.	Melocular Docking Scores		IC_{50}[a] (μM)		
	mTOR	PI3Kα	mTOR	PI3Kα	c-Met
10	9.58	9.05	0.86±0.10	0.69±0.10	>10
11	9.40	9.37	0.94±0.10	6.72±0.30	>10
24	6.27	6.96	6.26±0.52	>10	>10
27	7.92	8.57	9.43±0.80	4.23±0.20	>10
GDC-0941[b]	-	-	0.58	0.003	ND[c]
PI103[b]	-	-	0.019 ± 0.004	0.011 ± 0.002	ND

[a] The values are an average of two separate determinations.

[b] Used as a positive control

[c]ND: Not determined

(**Fig. 1**)

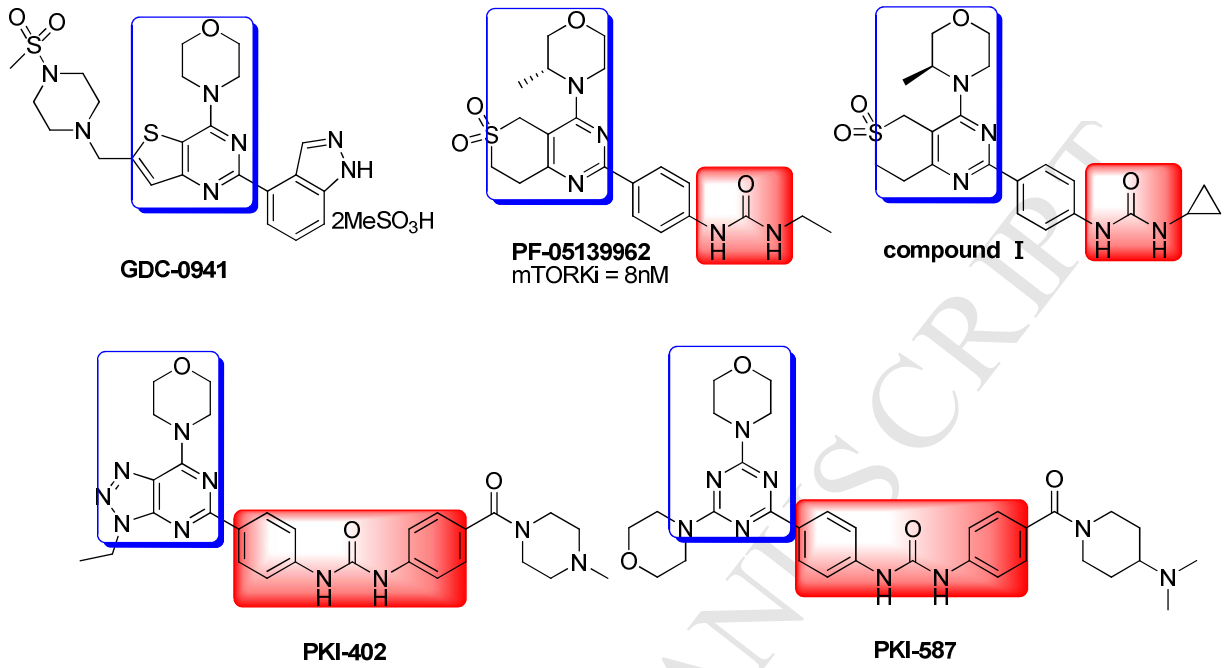


Fig.1. Structures of some reported (fused-)pyrimidine/triazines

(**Fig. 2**)

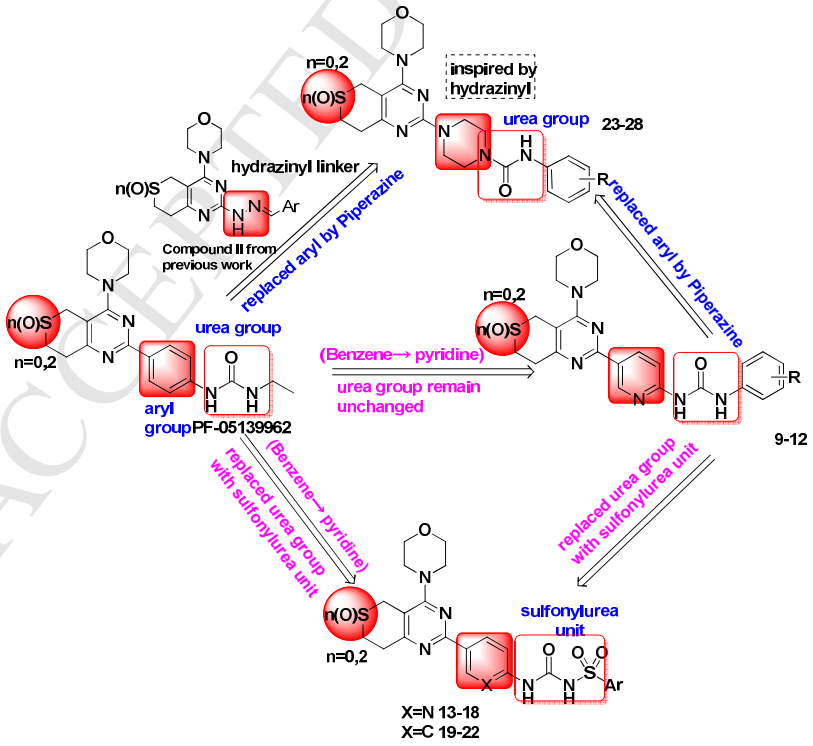


Fig.2. Structures and design strategy for target compounds **9**–**28**

(**Fig. 3.**)

Fig.3a-b

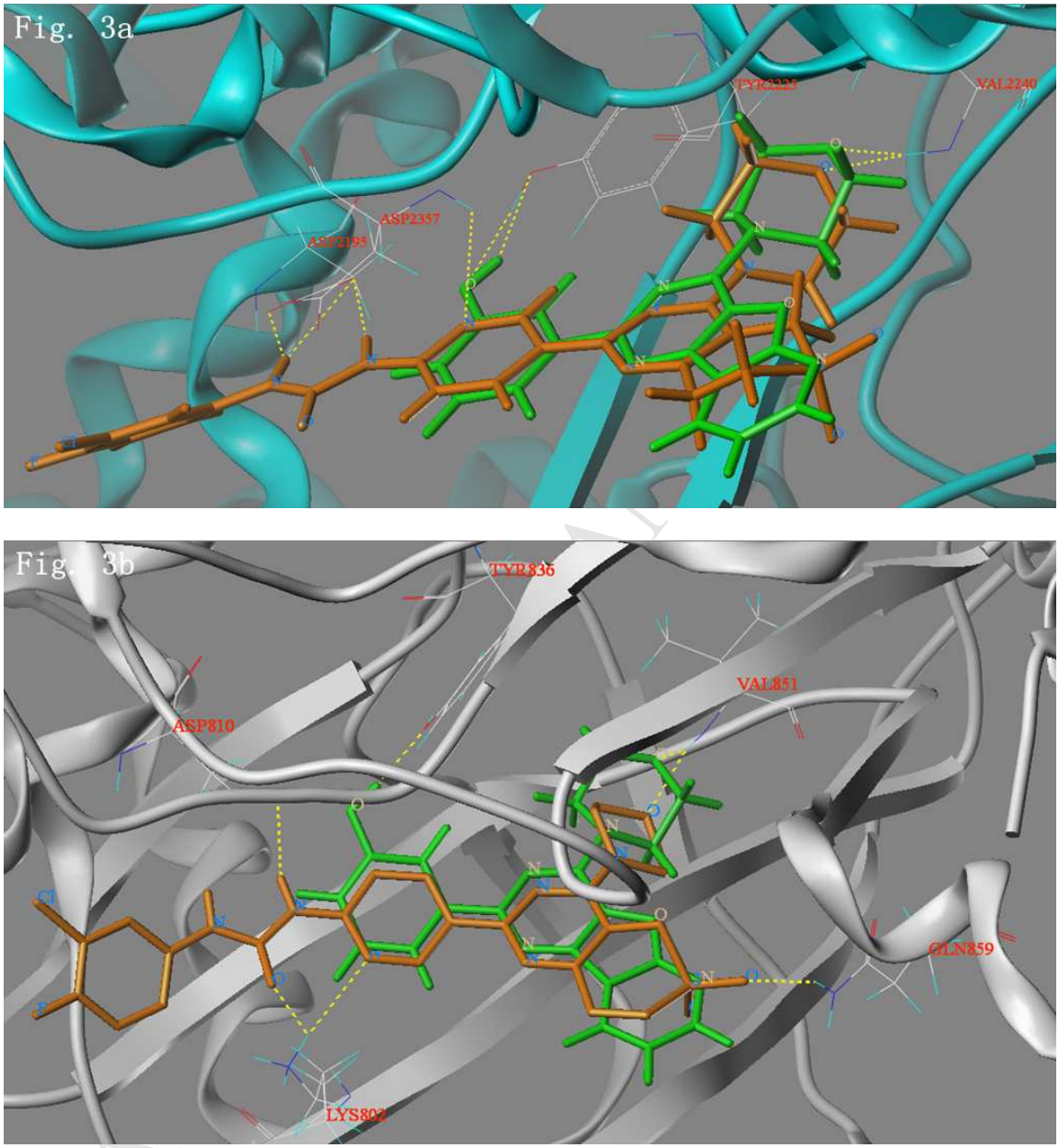
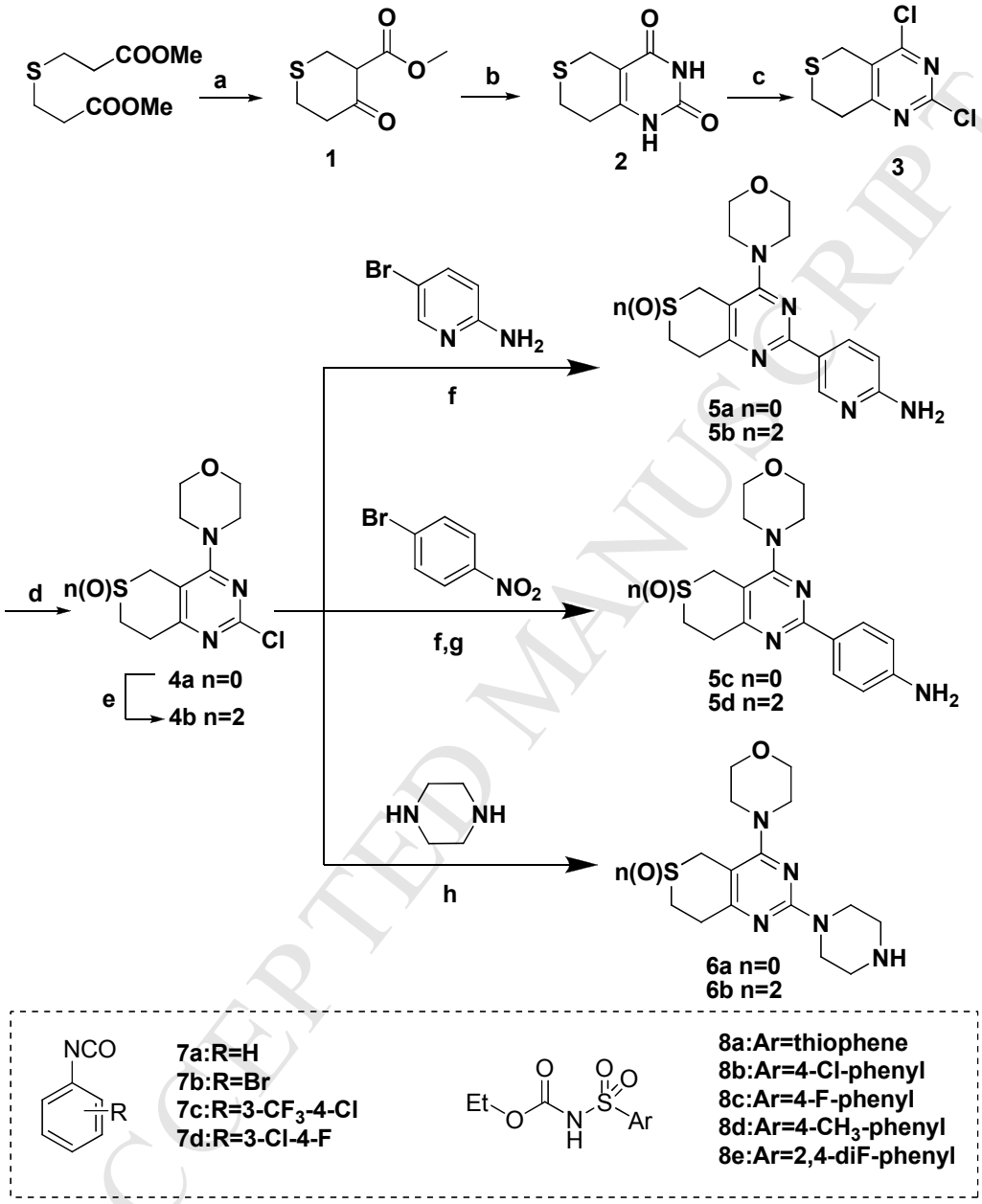


Fig. 3a-b. Binding models of compound **11** and native ligand PI 103 target into active site of mTOR(Fig.3a) and PI3Kα(Fig.3b). The proteins were displayed by cyan and white ribbon. Compound **11** and lead compound were displayed by orange and green sticks, respectively. H-bonding interactions between the **11**, lead compound and mTOR/PI3Kα were indicated with dashed lines in yellow.

(**Scheme 1.**)

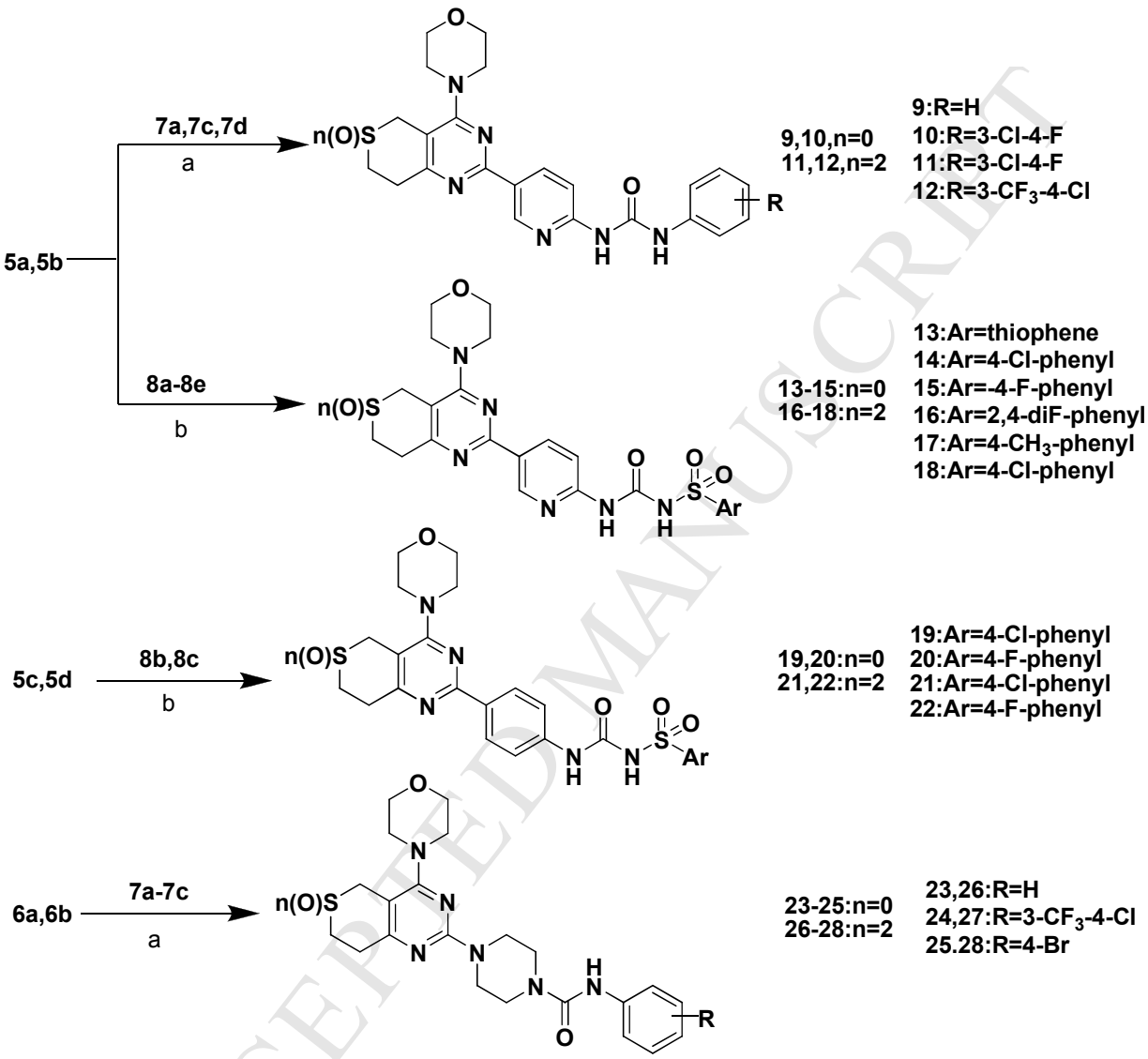
Scheme 1. Synthetic routes of key intermediates **5a**–**5d** and **6a**–**6b**



Reagents and conditions: (a) NaH, THF, rt, 3 h; (b) 3 equiv urea, C_2H_5ONa, EtOH, reflux, 24 h; (c) $POCl_3$, DMF(cat.), reflux, 3 h; (d) 2.4 equiv morpholine, MeOH, rt, 1.5 h; (e) $Na_2WO_4 \cdot 2H_2O$, 30% H_2O_2, 20℃, 3 h; (f) 1)bis(pinacolato)diboron, KAc, $Pd(PPh_3)Cl_2$, 1,2-dimethoxyethane, reflux, 2 h; 2) H_2O, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, reflux, 6 h; (g) 80% $NH_2NH_2 \cdot H_2O$, $FeCl_3.6H_2O$, actived C, EtOH, 78 ℃, 1 h; (h) EtOH, K_2CO_3, reflux, 2–3 h;

(**Scheme 2.**)

Scheme 2. Synthetic routes of target compounds **9**–**28**



Reagents and conditions: (a) 1,4-dioxane, rt, 10–30mins; (b) methylbenzene, rt, 4–8h.

Research Highlights

► Four series of 2-substituted-4-morpholino- 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives were designed and synthesized

► Most of the synthesized compounds showed moderate to significant antitumor activity.

►3-Cl-4-F (**10**–**11**) or 3-CF_3-4-Cl (**12**, **24**, **27**)substitution were more preferred.

► Compound **11** was a similar antitumor activities level of GDC-0941.

► Docking study was investigated to explore the binding modes of compounds with mTOR/PI3Ka