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Design, synthesis, anticancer activity and docking studies of novel 4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives as mTOR inhibitors

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ABSTRACT

A series of 7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine derivatives (**7a–q**, **10a–q**) were designed, synthesized and their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR, MS and HRMS spectrum. All the compounds were evaluated for the inhibitory activity against mTOR kinase at 10 μM level. Five selected compounds (**7b**, **7e**, **7h**, **10b** and **10e**) were further evaluated for the inhibitory activity against PI3Kα at 10 μM level, and the IC_{50} values against mTOR kinase and two cancer cell lines. Twelve of the target compounds exhibited moderate antitumor activities. The most promising compound **7e** showed strong antitumor activities against mTOR kinase, H460 and PC-3 cell lines with IC_{50} values of 0.80 ± 0.15 μM, 7.43 ± 1.45 μM and 11.90 ± 0.94 μM, which were 1.28 to 1.71-fold more active than BMCL-200908069-1 (1.37 ± 0.07 μM, 9.52 ± 0.29 μM, 16.27 ± 0.54 μM), respectively. Structure–activity relationships (SARs) and docking studies indicated that the thiopyrano[4,3-*d*]pyrimidine scaffolds exerted little effect on antitumor activities of target compounds. Substitutions of aryl group at C-4 position had a significant impact on the antitumor activities, and 4-OH substitution produced the best potency.

1. Introduction

The PI3K-Akt-mTOR signal pathway plays a key role in cell proliferation, migration, survival, and angiogenesis. Therefore, developing mTOR inhibitors is one of the research hotspots in molecular targeted therapy for the treatment of human cancer.[1,2]

In recent years, many (fused-)pyrimidine/triazine derivatives were reported as PI3K-Akt-mTOR signal pathway inhibitors (Fig. 1).[3–6] Compound BMCL-200908069-1 (Fig. 1) is a triazine-hydrazone derivative which exhibits potent anti-tumor activity with an mTOR IC_{50} value (half-maximal inhibitory concentration) of 0.27 μM.[6] Structure–activity relationships (SARs) showed that aryl hydrazones and morpholine-triazine moieties were essential for the mTOR activity. However, whether both of the two morpholine moieties were necessary remains indeterminate.

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 BMCL-200908069-1 were carried out in this research. Prompted by potent mTOR inhibitors reported recent years (Fig. 1),[3–6] we speculated that only one morpholine moiety was necessary, and the other one was fused to the triazine nucleus according to the theory of scaffold hopping. Meanwhile, we replaced the oxygen atom with sulfur atom and introduced different substituents to the aryl moiety, resulting in compounds **7a–q**. Further investigations were carried out in details to study the effect of oxidation of sulfur atom on the antitumor activity. Therefore, sulfur was oxidized to sulfone to afford compounds **10a–q**. The design strategy for all target compounds is shown in Figure 2.

Herein we disclosed the design, synthesis and antitumor activity against H460, PC-3 cancer cell lines, PI3Kα kinase and mTOR kinase of novel thiopyrano[4,3-*d*]pyrimidine derivatives. Moreover, docking studies were presented in this paper as well.

2. Chemistry

The preparation of target compounds **7a–q** and **10a–q** was described in Scheme 1.

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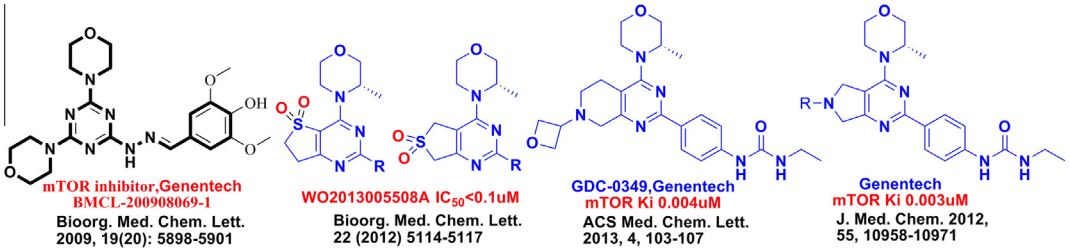


Figure 1. Structures of some reported (fused-)pyrimidine/triazines.

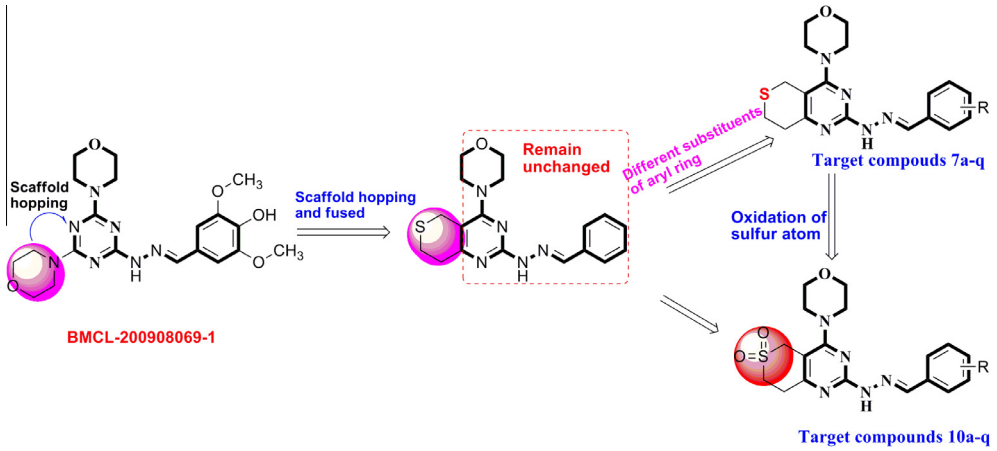


Figure 2. Structures and design strategy for target compounds **7a**–**q** and **10a**–**q**.

Compounds **7a**–**q** were synthesized from commercially available methyl dimethyl 3,3′-thiodipropionate through six steps. Intramolecular cyclization reaction of dimethyl 3,3′-thiodipropionate in the present of NaH (60%) afforded compound **1** as a yellow viscous oil. Subsequently, compound **1** was condensed with urea at reflux for 24 h to give thiopyrano[4,3-*d*]pyrimidine-2,4-dione (compound **2**). Chlorination of **2** with $POCl_3$ and DMF (cat.) for 3 h at 115 °C achieved **3** as yellow solid. Regioselective nucleophilic displacement of the 4-chloride with morpholine, followed by hydrazinolysis gave access to the key intermediate **5**. Finally, **5** condensed with substituted aromatic aldehydes **6a**–**q** to furnish target compounds **7a**–**q**, respectively.

On the other hand, compound **4** treated with sodium tungstate dihydrate and 30% hydrogen peroxide to furnish oxidate **8** which was then used in a substitution reaction with $NH_2NH_2{\cdot}H_2O$ (80%) to generate another key intermediate **9**. Similarly, intermediate **9** condensed with the corresponding aromatic aldehydes **6a**–**q** to afford target compounds **10a**–**q**, respectively.

The relative stereochemistry of the target compounds (**7a**–**q**, **10a**–**q**) was easily confirmed as *E* isomeric form according to the method in our previous researches.[7–9]

3. Results and discussion

3.1. Biological evaluation

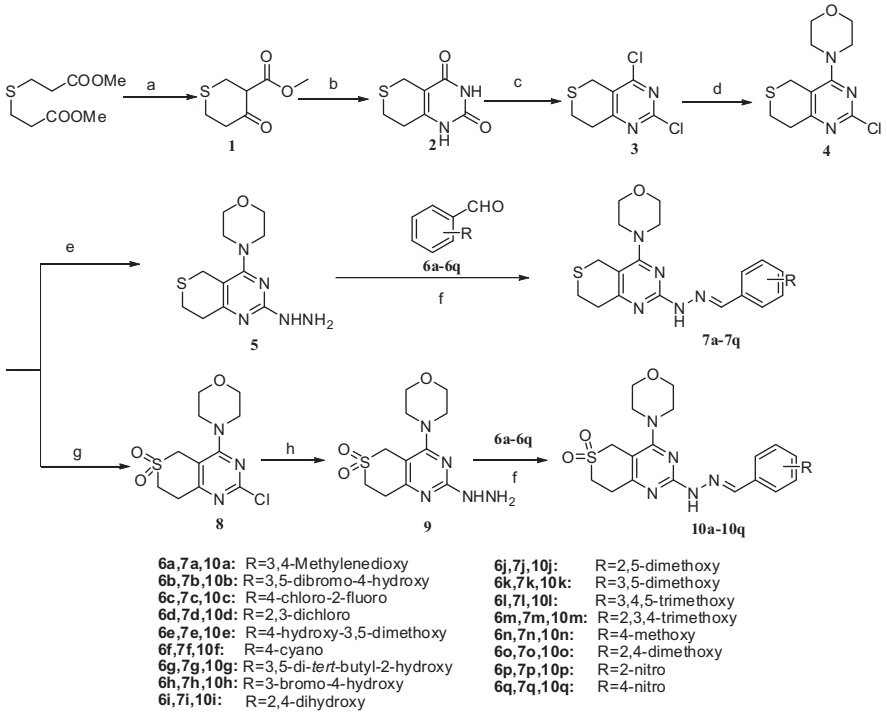
The target compounds (**7a**–**q** and **10a**–**q**) were evaluated for their inhibitory activity against mTOR kinase at 10 μM level in vitro together with reference compounds BMCL200908069-1 and PI103 by LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay. In addition, the five selected compounds (**7b**, **7e**, **7h**, **10b** and **10e**) were further evaluated for the inhibitory activity against PI3Kα at 10 μM level using Kinase-Glo® Luminescent Kinase assay, and the IC_{50} values against mTOR kinase and two cancer cell lines H460 (human lung cancer) and PC-3 (human prostate cancer). The results expressed as inhibition rates or IC_{50} values were summarized in Tables 1 and 2 and the values are the average of at least two independent experiments.

As illustrated in Tables 1 and 2, twelve of the synthesized compounds showed moderate to excellent inhibitory activity on mTOR kinase. Five of them (**7b**, **7e**, **7h**, **10b**, **10e**) showed excellent inhibitory activity on mTOR kinase with the inhibition rates from 64.1 ± 2.8% to 89.5 ± 1.8% (IC_{50} values from 6.93 ± 0.54 μM to 0.80 ± 0.03 μM), especially compound **7e** was 1.71-fold more active than lead compound BMCL-200908069-1.

The results suggested that replacement of the triazine scaffold with thiopyrano[4,3-*d*]pyrimidine scaffold enhanced the antitumor activity. It also told us that not both the two morpholine moieties were necessary and the application of scaffold hopping was feasible. The oxidation of sulfur atom had little effect on the antitumor activity, as we could not find significant improved in antitumor activity of compounds **10a**–**q**.

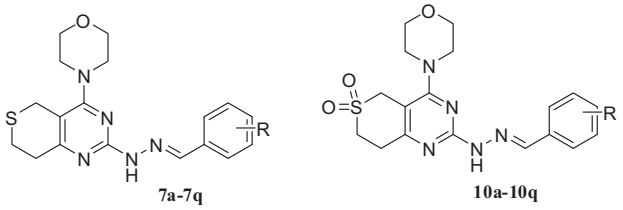
The inhibitory activities on mTOR kinase of compounds **7a**–**q** were increased in varying degrees because of the different substituents of aryl moiety. It was noticeable that hydroxyl group at C-4 position significantly increased the activity, such as compounds **7e** and **10e** with the antitumor activity equal to the positive control BMCL-200908069-1. This dramatic boost in the mTOR potency by the hydroxyl group could be ascribed to the increased hydrogen bonds between the 4-OH and receptors necessary for activity. The results showed that variations in substitution at 4-position of the aryl moiety had a marked impact on the activity. For compounds **7a**–**q** and **10a**–**q**, 4-OH substitution of the aryl moiety was more preferred.

The antitumor activities of the five selected compounds (**7b**, **7e**, **7h**, **10b** and **10e**) as well as the two lead compounds against PI3Kα,



Scheme 1. Synthetic routes of target compounds **7a–q** and **10a–q**. 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) 80% $NH_2NH_2{\cdot}H_2O$, 85 °C, 1 h; (f) EtOH, reflux, 1–3 h; (g) $Na_2WO_4{\cdot}2H_2O$, 30% H_2O_2, 20 °C, 3 h; (h) 80% $NH_2NH_2{\cdot}H_2O$, EtOH, 75 °C, 1 h.

Table 1
Structures and of compounds **7a–q**, **10a–q**



Compounds No.	R	mTOR[a] (10 μM inhibitory %)	Compounds No.	R	mTOR[a] (10 μM inhibitory %)
7a	3,4-Methylenedioxy	NA[c]	**10a**	3,4-Methylenedioxy	44.8 ± 0.3
7b	3,5-Dibromo-4-hydroxy	78.2 ± 0.6	**10b**	3,5-Dibromo-4-hydroxy	64.1 ± 4.4
7c	4-Chloro-2-fluoro	NA	**10c**	4-Chloro-2-fluoro	NA
7d	2,3-dichloro	NA	**10d**	2,3-Dichloro	NA
7e	4-Hydroxy-3,5-dimethoxy	89.5 ± 1.8	**10e**	4-Hydroxy-3,5-dimethoxy	76.6 ± 2.2
7f	4-Cyano	61.0 ± 4.5	**10f**	4-Cyano	44.2 ± 6.9
7g	3,5-Di-*tert*-butyl-2-hydroxy	NA	**10g**	3,5-Di-*tert*-butyl-2-hydroxy	NA
7h	3-Bromo-4-hydroxy	70.4 ± 2.8	**10h**	3-Bromo-4-hydroxy	56.9 ± 5.8
7i	2,4-Dihydroxy	54.0 ± 4.0	**10i**	2,4-Dihydroxy	44.6 ± 7.2
7j	2,5-Dimethoxy	29.3 ± 3.2	**10j**	2,5-Dimethoxy	10.9 ± 4.3
7k	3,5-Dimethoxy	39.7 ± 3.3	**10k**	3,5-Dimethoxy	33.6 ± 4.7
7l	3,4,5-Trimethoxy	11.8 ± 15.3	**10l**	3,4,5-Trimethoxy	13.2 ± 4.9
7m	2,3,4-Trimethoxy	10.3 ± 1.8	**10m**	2,3,4-Trimethoxy	NA
7n	4-Methoxy	12.5 ± 2.4	**10n**	4-Methoxy	20.3 ± 7.1
7o	2,4-Dimethoxy	43.7 ± 2.9	**10o**	2,4-Dimethoxy	44.6 ± 3.2
7p	2-Nitro	NA	**10p**	2-Nitro	NA
7q	4-Nitro	NA	**10q**	4-Nitro	13.6 ± 6.4
BMCL-200908069-1[b]	—	76.1 ± 0.3	PI-103[b]	—	98.6 ± 0.5

[a] The values are an average of two separate determinations.
[b] Used as a positive control.
[c] NA: not active (inhibitory rate <10%).

mTOR kinase and two cancer cell lines were shown in Table 2. Obviously, the activities of these five compounds were equal to BMCL-200908069-1, or even better, which further confirmed their strong antitumor activities. However, all these compounds showed bad inhibitory activity against PI3Kα. The most promising compound **7e** showed strong antitumor activities against mTOR kinase,

Table 2
PI3Kα/mTOR kinase activity and cytotoxicity of selected compounds and positive controls

Compounds No.	IC_{50}[a] (μM)			
	mTOR	PI3Kα (10 μM inhibitory %)	H460	PC-3
7b	2.67 ± 1.32	21.6 ± 7.0	ND[c]	>20
7e	0.80 ± 0.15	12.0 ± 5.5	7.43 ± 1.45	11.90 ± 0.94
7h	1.94 ± 1.15	18.8 ± 1.9	10.75 ± 3.19	14.4 ± 1.69
10b	6.93 ± 0.63	24.9 ± 7.6	ND[c]	>20
10e	2.20 ± 0.77	6.2 ± 3.5	ND[c]	>20
BMCL-200908069-1[b]	1.37 ± 0.07	—	9.52 ± 0.29	16.27 ± 0.54
PI-103[b]	0.019 ± 0.004	0.011 ± 0.002 (IC_{50})	ND[c]	ND[c]

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

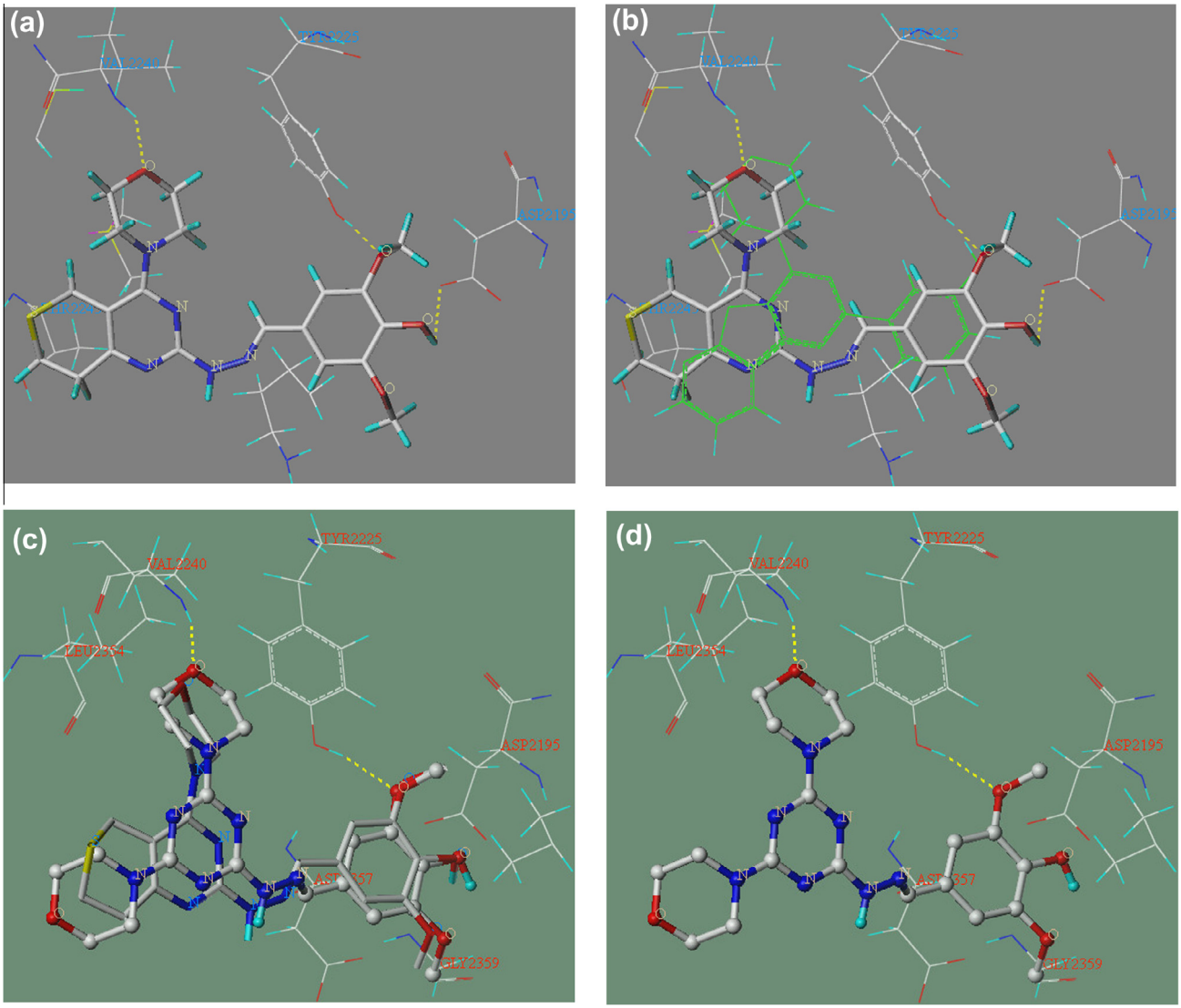


Figure 3. Binding model of compound **7e** target into active site of mTOR and hydrogen bonds were showed in dashed lines (yellow). Compounds (**3a**–**3d**): binding model of compound **7e** (shown in Capped Sticks), BMCL-200908069-1 (shown in Ball and Stick) and native ligand PI103 (shown in yellow) with mTOR, repectively.

H460 and PC-3 cell lines with IC_{50} values of 0.80 ± 0.15 μM, 7.43 ± 1.45 μM and 11.90 ± 0.94 μM, which were 1.28 to 1.71-fold more active than BMCL-200908069-1 (1.37 ± 0.07 μM, 9.52 ± 0.29 μM, 16.27 ± 0.54 μM), respectively.

In view of above, thiopyrano[4,3-*d*]pyrimidine scaffolds exerted little effect on the antitumor activities of these thiopyrano[4,3-*d*]pyrimidine derivatives. Variations in substitutions of the aryl moiety had a significant impact on the activity, and 4-OH substitution was the most preferred.

3.2. Molecular docking study

To explore the binding modes of target compounds with the active site of mTOR, 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 compound **7e**, our best mTOR inhibitor in this study, as ligand example, and the structure of mTOR was selected as the docking model (PDB ID code: 4JT6[10]).

The binding modes of compounds **7e** and lead compounds were shown in Figure 3a–d. As depicted in Figure 3a–d, compound **7e** and BMCL-200908069-1 can nearly overlap in the binding model and morpholine group, methoxy group, hydroxyl group formed three hydrogen bonds with residues VAL2240, TYR2225, ASP2195, respectively. The H-bond distances and donor-H-acceptor angles are 2.107 Å, 144.60° and 2.146 Å, 173.61° and 2.362 Å, 82.82°, respectively. The results showed that the quality of these three H-bonds is well. Analysis of compound **7e**'s binding mode in the active binding site demonstrated that the docking mode of the **7e** is similar to the lead compound BMCL-200908069-1 and native ligand PI-103 with the same H-bond between morpholine group and residues VAL2240. The three hydrogen bonds really play an important role in increasing the inhibitory potency of thiopyrano[4,3-*d*]pyrimidine derivatives against mTOR kinase according to the docking results and the activity. Furthermore, the docking results also give us a new direction to design new mTOR inhibitors that can interact with VAL2240, TYR2225 and ASP2195. The above-mentioned results of SAR analysis and molecular docking study may allow the rational design of more potent mTOR inhibitors.

4. Conclusions

In summary, a series of thiopyrano[4,3-*d*]pyrimidine derivatives were designed, synthesized and evaluated for antitumor activity against mTOR kinase and two cancer cell lines in vitro. The pharmacological results indicated that twelve of the synthesized compounds displayed moderate antitumor activity and five selected compounds showed equal to more potency than lead compound BMCL-200908069-1. The most promising compound **7e** exhibited strong antitumor activities against mTOR kinase, H460 and PC-3 cell lines, which were 1.28 to 1.71-fold more active than BMCL-200908069-1. The initial SARs and docking studies showed that the thiopyrano[4,3-*d*]pyrimidine scaffold had little effect on the antitumor activities, while variations in substitutions of the aryl moieties had a significant impact on the activities and 4-OH substitution produced the best potency. Further studies 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 LC–MS (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 specified. Yields were not optimized.

5.2. Preparation of methyl 4-oxotetrahydro-2*H*-thiopyran-3-carboxylate (1)

To the mixture of NaH (60%, 8.19 g, 0.205 mol) and anhydrous THF (100 mL), a solution of dimethyl 3,3′-thiodipropionate (31.8 g, 0.154 mol) in anhydrous THF (40 mL) was added via a dropping funnel over 1 h (the dropping funnel was rinsed with 15 mL of THF) at room temperature under N_2. After stirring for 2 h at rt, the reaction was completed by TLC analysis (20% EtOAc in light petroleum (60–90 °C). The mixture was transferred to a beaker, dilute hydrochloric acid was added slowly with stirring maintaining the temperature below 20 °C; the final pH was 6–7. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), then dried over Na_2SO_4, the filtrate was concentrated under reduced pressure to afford **1** as a yellow viscous oil (26.8 g, 99.8%) and was used for next step without further purification. ESI-MS *m*/*z*: $[M+H]^+$ 175.2.

5.3. Preparation of 7,8-dihydro-1*H*-thiopyrano[4,3-*d*]pyrimidine-2,4(3*H*,5*H*)-dione (2)

Finely cut sodium metal (16.2 g, 0.704 mol) was added into 300 mL anhydrous ethanol at ice bath with stirring. After the sodium metal was completely consumed, urea (32.7 g, 0.545 mol) and methyl 4-oxotetrahydro-2*H*-thiopyran-3-carboxylate **1** (31.3 g, 0.180 mol) was added to the solution. The mixture was heated and refluxed for 24 h, and the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and the residue was poured into ice water, then adjusted the pH to 6–7 with acetic acid under ice bath, filtration, the filter cake was washed with ice-water, dried to obtain **2** as white powdery solid (13.6 g, 41.2%). Mp >300 °C. ESI-MS *m*/*z*: $[M-H]^-$ 183.2, ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 10.75 (s, 1H), 3.39 (s, 2H), 2.80 (t, *J* = 5.8 Hz, 2H), 2.57 (t, *J* = 5.6 Hz, 2H).

5.4. Preparation of 2,4-dichloro-7,8-dihydro-5*H*-thiopyrano-[4,3-*d*]pyrimidine (3)

A mixture of 7,8-dihydro-1*H*-thiopyrano[4,3-*d*]pyrimidine-2,4(3*H*,5*H*)-dione **2** (3.03 g, 0.016 mol), $POCl_3$ (20 mL) was heated at reflux for 3 h and the reaction was monitored by TLC. The reaction mixture was slowly added to ice/water with vigorous stirring yielding a precipitate. The mixture was then filtered to yield 2,4-dichloro-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine **3** as yellow solid (3.12 g, 85.7%). Mp 87.1–87.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 2H), 3.23 (t, *J* = 5.9 Hz, 2H), 2.96 (t, *J* = 6.0 Hz, 2H).

5.5. Preparation of 4-(2-chloro-7,8-dihydro-5*H*-thiopyrano-[4,3-*d*]pyrimidin-4-yl)morpholine (4)

To the mixture of 2,4-dichloro-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine **3** (7.40 g, 0.033 mol) and MeOH (150 mL), morpholine (7 mL, 0.08 mol) was added drop-wise at room temperature. The reaction mixture then was stirred at room temperature for 1.5 h. After completion of reaction as indicated by TLC, the mixture was then filtered, washed with water and MeOH, to yield the title compound as white solid (6.84 g, 75.3%). Mp 161.1–162.4 °C. ESI-MS *m*/*z*: $[M+H]^+$ 272.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 2H), 3.23 (t, *J* = 5.9 Hz, 2H), 2.96 (t, *J* = 6.0 Hz, 2H).

5.6. Preparation of 4-(2-hydrazinyl-7,8-dihydro-5*H*-thiopyrano-[4,3-*d*]pyrimidin-4-yl)morpholine (5)

A solution of 80% hydrazine hydrate ($NH_2NH_2{\cdot}H_2O$, 110 mL) and compound **4** (3.47 g, 0.013 mol) was stirred at 85 °C for 1 h. The precipitate was collected by filtration and washed with water, and dried to give **5** as white solid (1.73 g, 50.7%). Mp 172.1–173.2 °C. ESI-MS *m*/*z*: $[M+H]^+$ 268.2. ^{1}H NMR (400 MHz, DMSO) δ 7.66 (s, 1H), 4.07 (s, 2H), 3.73–3.66 (m, 4H), 3.54 (s, 2H), 3.18 (s, 4H), 2.89 (t, *J* = 6.1 Hz, 2H), 2.82 (t, *J* = 6.0 Hz, 2H).

5.7. Preparation of 2-chloro-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (8)

Compound **4** (4.03 g, 0.015 mol) was first dissolved in methanol (60 mL), sodium tungstate dihydrate (0.447 g, 1.36 mmol) and 30% hydrogen peroxide (5.0 mL, 0.048 mol) were successively dropped into the solution and stirred at 20 °C for 3 h. Then, the reaction mixture was filtered, washed with acetone, dried and gave compound **8** as pale yellow solid (3.92 g, 87.0%). Mp 145.7–146.8 °C. ESI-MS *m*/*z*: $[M+Na]^+$ 326.1, $[M-H]^-$ 302.1. ^{1}H NMR (400 MHz, DMSO) δ 4.38 (s, 2H), 3.77–3.69 (m, 4H), 3.56 (t, J = 6.6 Hz, 2H), 3.45–3.39 (m, 4H), 3.31 (t, J = 6.7 Hz, 2H).

5.8. Preparation of 2-hydrazinyl-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (9)

The synthesis of compound **9** was similar to the compound **5**. A mixture of 80% hydrazine hydrate ($NH_2NH_2{\cdot}H_2O$, 70 mL), C_2H_5OH (70 mL) and compound **8** (3.40 g, 0.011 mol) was stirred at 75 °C for 1 h. The precipitate was collected by filtration and washed with water, and dried to give **9** as white solid (2.54 g, 75.7%). Mp 230.2–231.9 °C. ESI-MS *m*/*z*: $[M+H]^+$ 300.1. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (s, 1H), 4.13 (s, 2H), 4.10 (s, 2H), 3.70 (s, 4H), 3.43 (t, J = 6.4 Hz, 2H), 3.17 (s, 4H), 3.11 (t, J = 6.7 Hz, 2H).

5.9. General procedure for the preparation of compounds 7a–7q and 10a–10q

A mixture of 4-(2-hydrazinyl-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine **5** (53 mg, 0.20 mmol) or 2-hydrazinyl-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide **9** (60 mg, 0.20 mmol), substituted aryl aldehydes **6a–q** (0.20 mmol) and one drop of glacial acetic acid in absolute ethanol (10 mL) was refluxed for 1–3 h. The mixture was cooled, separated by filtration and washed with EtOH to afford the solids **7a–q** and **10a–q**.

5.9.1. (*E*)-4-(2-(2-(Benzo[*d*][1,3]dioxol-5-ylmethylene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7a)

This compound was obtained as white solid in 82% yield. Mp 243.6–244.8 °C. ESI-MS *m*/*z*: $[M+H]^+$ 400.2. ^{1}H NMR (400 MHz, DMSO) δ 10.67 (s, 1H), 8.00 (s, 1H), 7.21 (s, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 6.05 (s, 2H), 3.72 (s, 4H), 3.59 (s, 2H), 3.27–3.22 (m, 4H), 2.91 (s, 4H).

5.9.2. (*E*)-2,6-Dibromo-4-((2-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)phenol (7b)

This compound was obtained as yellow solid in 85% yield. Mp 166.8–171.5 °C. ESI-MS *m*/*z*: $[M+H]^+$ 529.9. ^{1}H NMR (400 MHz, DMSO) δ 10.91 (s, 1H), 7.97 (s, 1H), 7.80 (s, 2H), 4.35 (s, 1H), 3.74 (s, 4H), 3.62 (s, 2H), 3.29 (s, 4H), 2.95 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.51, 138.00, 137.86, 130.10(2C), 112.81(2C), 109.84, 66.46(2C), 49.38(2C), 33.83, 26.23, 26.14. ESI-HRMS *m*/*z*: calcd for $C_{18}H_{19}Br_2N_5O_2S$ $[M+H]^+$: 527.9704; found 527.9689, 529.9667, 531.9645.

5.9.3. (*E*)-4-(2-(2-(2-Chloro-4-fluorobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7c)

This compound was obtained as yellow solid in 83% yield. Mp 234.1–236.9 °C. ESI-MS *m*/*z*: $[M+H]^+$ 408.1. ^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.44 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 9.3 Hz, 1H), 7.30 (s, 1H), 3.72 (s, 4H), 3.60 (s, 2H), 3.27 (s, 4H), 2.92 (s, 4H).

5.9.4. (*E*)-4-(2-(2-(2,3-Dichlorobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7d)

This compound was obtained as yellow solid in 82% yield. Mp 239.3–241.3 °C. ESI-MS *m*/*z*: $[M+H]^+$ 424.1. ^{1}H NMR (400 MHz, DMSO) δ 11.28 (s, 1H), 8.51 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 3.73 (s, 4H), 3.61 (s, 2H), 3.28 (s, 4H), 2.93 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.85, 165.55, 157.26, 136.15, 135.69, 132.67, 130.44, 130.12, 128.71, 125.25, 110.35, 66.47(2C), 49.37(2C), 33.87, 26.23(2C).

5.9.5. (*E*)-2,6-Dimethoxy-4-((2-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)phenol (7e)

This compound was obtained as yellow solid in 75% yield. Mp 141.6–146.2 °C. ESI-MS *m*/*z*: $[M+H]^+$ 432.2. ^{1}H NMR (400 MHz, DMSO) δ 10.74 (s, 1H), 8.67 (s, 1H), 7.95 (s, 1H), 6.89 (s, 2H), 3.80 (s, 6H), 3.71 (s, 4H), 3.59 (s, 2H), 3.44–3.30 (m, 4H), 2.91 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 166.83, 166.83, 162.40, 160.03, 159.62, 157.87, 144.03, 131.41, 111.58, 107.70, 103.28, 66.35(2C), 49.34, 49.15(2C), 46.95, 32.34. ESI-HRMS *m*/*z*: calcd for $C_{20}H_{25}N_5O_4S$ $[M+H]^+$: 432.1706; found 432.1686.

5.9.6. (*E*)-4-((2-(4-Morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)benzonitrile (7f)

This compound was obtained as yellow solid in 84% yield. Mp 281.6–283.2 °C. ESI-MS *m*/*z*: $[M+H]^+$ 381.1. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (s, 1H), 8.10 (s, 1H), 7.82 (dd, J = 24.4, 8.1 Hz, 4H), 3.77 (d, J = 32.9 Hz, 4H), 3.61 (s, 2H), 3.28 (s, 4H), 2.93 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.84, 165.57, 157.26, 140.56, 138.44(2C), 133.10(2C), 127.03, 119.45, 110.68, 110.35, 66.48(2C), 49.37(2C), 33.86, 26.23(2C).

5.9.7. (*E*)-2,6-Di-*tert*-butyl-4-((2-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)phenol (7g)

This compound was obtained as pale yellow solid in 83% yield. Mp 147.5–150.3 °C. ESI-MS *m*/*z*: $[M+H]^+$ 484.2. ^{1}H NMR (400 MHz, DMSO) δ 10.68 (s, 1H), 7.96 (s, 1H), 7.41 (s, 2H), 7.20 (s, 1H), 3.71 (s, 4H), 3.57 (d, J = 13.3 Hz, 2H), 3.29–3.22 (m, 4H), 2.90 (d, J = 6.2 Hz, 4H), 1.39 (d, J = 10.7 Hz, 18H).

5.9.8. (*E*)-2-Bromo-4-((2-(4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)phenol (7h)

This compound was obtained as pale yellow solid in 88% yield. Mp 292.6–294.6 °C. ESI-MS *m*/*z*: $[M+H]^+$ 452.1. ^{1}H NMR (400 MHz, DMSO) δ 10.76 (s, 1H), 10.58 (s, 1H), 7.94 (d, J = 10.9 Hz, 1H), 7.74 (s, 1H), 7.44 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 3.72 (s, 4H), 3.59 (s, 2H), 3.25 (s, 4H), 2.92 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.69, 165.56, 157.55, 155.07, 139.57, 130.74, 128.76, 127.37, 116.98, 110.23, 109.53, 66.48(2C), 49.41(2C), 33.85, 26.27, 26.14. ESI-HRMS *m*/*z*: calcd for $C_{18}H_{20}BrN_5O_2S$ $[M+H]^+$: 450.0599; found 450.0580,452.0588.

5.9.9. (*E*)-4-((2-(4-Morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)benzene-1,3-diol (7i)

This compound was obtained as yellow solid in 78% yield. Mp 252.5–253.7 °C. ESI-MS *m*/*z*: $[M+H]^+$ 388.2. ^{1}H NMR (400 MHz, DMSO) δ 12.14 (s, 1H), 11.04 (s, 1H), 9.76 (s, 1H), 8.08 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 6.30 (d, J = 8.4 Hz, 1H), 6.28 (s, 1H), 3.72 (s, 4H), 3.59 (s, 2H), 3.28 (s, 4H), 2.90 (dd, J = 14.9, 5.1 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.78, 165.54, 159.81, 159.55, 157.07, 143.16, 131.21, 111.72, 109.40, 107.59, 103.28, 66.48(2C), 49.30(2C), 33.85, 26.21, 26.17.

5.9.10. (*E*)-4-(2-(2-(2,5-Dimethoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7j)

This compound was obtained as yellow solid in 86% yield. Mp 133.5–135.9 °C. ESI-MS *m*/*z*: $[M+H]^+$ 416.2. ^{1}H NMR (400 MHz,

DMSO) δ 10.93 (s, 1H), 8.37 (s, 1H), 7.37 (s, 1H), 6.99 (d, J = 8.9 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 3.81 (d, J = 24.8 Hz, 4H), 3.73 (d, J = 6.6 Hz, 6H), 3.59 (s, 2H), 3.27 (s, 4H), 2.92 (s, 4H).

5.9.11. (*E*)-4-(2-(2-(3,5-Dimethoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7k)

This compound was obtained as pale yellow solid in 79% yield. Mp 252.2–254.2 °C. ESI-MS m/z: $[M+H]^+$ 416.2. ^{1}H NMR (400 MHz, DMSO) δ 10.71 (s, 1H), 8.32 (s, 1H), 7.74 (d, J = 9.0 Hz, 1H), 6.59 (s, 2H), 3.85 (dd, J = 32.9, 12.8 Hz, 6H), 3.72 (s, 4H), 3.59 (s, 2H), 3.24 (s, 4H), 2.90 (d, J = 5.2 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.65, 165.55, 161.71, 158.66, 157.73, 136.60, 126.58, 116.94, 109.19, 106.64, 98.66, 66.49(2C), 56.15, 55.81, 49.42(2C), 33.84, 26.29, 26.12.

5.9.12. (*E*)-4-(2-(2-(3,4,5-Trimethoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7l)

This compound was obtained as pale yellow solid in 74% yield. Mp 111.6–113.8 °C. ESI-MS m/z: $[M+H]^+$ 446.2. ^{1}H NMR (400 MHz, DMSO) δ 10.91 (s, 1H), 7.98 (s, 1H), 6.93 (s, 2H), 3.81 (s, 6H), 3.71 (s, 4H), 3.68 (s, 3H), 3.59 (s, 2H), 3.29 (s, 4H), 2.92 (s, 4H).

5.9.13. (*E*)-4-(2-(2-(2,3,4-Trimethoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7m)

This compound was obtained as pale yellow solid in 73% yield. Mp 120.5–124.6 °C. ESI-MS m/z: $[M+H]^+$ 446.2. ^{1}H NMR (400 MHz, DMSO) δ 10.77 (s, 1H), 8.27 (s, 1H), 7.54 (d, J = 8.9 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 3.85–3.75 (m, 9H), 3.72 (s, 4H), 3.59 (s, 2H), 3.25 (s, 4H), 2.91 (s, 4H).

5.9.14. (*E*)-4-(2-(2-(4-Methoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7n)

This compound was obtained as yellow solid in 77% yield. Mp 120.5–124.6 °C. ESI-MS m/z: $[M+H]^+$ 386.2. ^{1}H NMR (400 MHz, DMSO) δ 10.72 (s, 1H), 8.03 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 6.98 (d, J = 8.3 Hz, 2H), 3.79 (s, 3H), 3.73 (s, 4H), 3.60 (s, 2H), 3.25 (s, 4H), 2.92 (dd, J = 7.4, 4.3 Hz, 4H).

5.9.15. (*E*)-4-(2-(2-(2,4-Dimethoxybenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7o)

This compound was obtained as white solid in 87% yield. Mp 248.6–249.2 °C. ESI-MS m/z: $[M+H]^+$ 416.2. ^{1}H NMR (400 MHz, DMSO) δ 10.71 (s, 1H), 8.32 (s, 1H), 7.75 (d, J = 5.8 Hz, 1H), 6.60 (s, 2H), 3.81 (d, J = 11.0 Hz, 6H), 3.72 (s, 4H), 3.59 (s, 2H), 3.25 (s, 4H), 3.00–2.84 (m, 4H).

5.9.16. (*E*)-4-(2-(2-(2-Nitrobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7p)

This compound was obtained as orange-yellow solid in 89% yield. Mp 259.3–260.8 °C. ESI-MS m/z: $[M+H]^+$ 401.2. ^{1}H NMR (400 MHz, DMSO) δ 11.30 (s, 1H), 8.48 (s, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 3.73 (s, 4H), 3.61 (s, 2H), 3.29 (s, 4H), 2.93 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 165.84, 165.49, 157.25, 147.98, 135.18, 133.75, 130.08, 129.55, 127.84, 124.97, 110.35, 66.48(2C), 49.34(2C), 33.87, 26.23(2C).

5.9.17. (*E*)-4-(2-(2-(4-Nitrobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine (7q)

This compound was obtained as orange-yellow solid in 86% yield. Mp 268.6–269.8 °C. ESI-MS m/z: $[M+H]^+$ 401.2. ^{1}H NMR (400 MHz, DMSO) δ 11.30 (s, 1H), 8.26 (d, J = 8.8 Hz, 2H), 8.17 (s, 1H), 7.87 (d, J = 8.8 Hz, 2H), 3.74 (d, J = 3.7 Hz, 4H), 3.62 (s, 2H), 3.30 (d, J = 4.0 Hz, 4H), 2.94 (s, 4H).

5.9.18. (*E*)-2-(2-(Benzo[*d*][1,3]dioxol-5-ylmethylene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10a)

This compound was obtained as pale yellow solid in 79% yield. Mp 285.3–286.0 °C. ESI-MS m/z: $[M+H]^+$ 432.1. ^{1}H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 8.02 (s, 1H), 7.23 (s, 1H), 7.06 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.07 (s, 2H), 4.20 (s, 2H), 3.73 (d, J = 4.4 Hz, 4H), 3.48 (s, 2H), 3.24 (s, 4H), 3.20 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.88, 162.33, 158.42, 148.57, 148.33, 141.52, 130.24, 122.38, 108.89, 105.19, 102.86, 101.80, 66.34(2C), 49.35, 49.25(2C), 47.02, 32.35.

5.9.19. (*E*)-2-(2-(3,5-Dibromo-4-hydroxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10b)

This compound was obtained as white solid in 68% yield. Mp 276.4–277.1 °C. ESI-MS m/z: $[M-H]^-$ 559.9. ^{1}H NMR (400 MHz, DMSO) δ 11.14 (s, 1H), 10.27 (s, 1H), 7.96 (s, 1H), 7.80 (s, 2H), 4.21 (s, 2H), 3.73 (s, 4H), 3.46 (d, J = 6.9 Hz, 2H), 3.24 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 166.82, 162.38, 158.23, 151.59, 138.78, 130.44, 130.25(2C), 112.76(2C), 103.30, 66.32(2C), 49.31, 49.23(2C), 46.99, 32.38. ESI-HRMS m/z: calcd for $C_{18}H_{19}Br_2N_5O_4S$ $[M+H]^+$: 559.9603; found 561.9567, 562.0833.

5.9.20. (*E*)-2-(2-(2-Chloro-4-fluorobenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10c)

This compound was obtained as white solid in 90% yield. Mp 262.3–262.9 °C. ESI-MS m/z: $[M-H]^-$ 438.1. ^{1}H NMR (400 MHz, DMSO) δ 11.35 (s, 1H), 8.46 (s, 1H), 8.07–7.95 (m, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 4.22 (s, 2H), 3.73 (s, 4H), 3.48 (t, J = 6.4 Hz, 2H), 3.31–3.15 (m, 6H).

5.9.21. (*E*)-2-(2-(2,3-Dichlorobenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10d)

This compound was obtained as yellow solid in 87% yield. Mp 275.8–276.9 °C. ESI-MS m/z: $[M-H]^-$ 454.1. ^{1}H NMR (400 MHz, DMSO) δ 11.50 (s, 1H), 8.56 (s, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.66 (d, J = 7.9 Hz, 1H), 7.45 (t, J = 8.3 Hz, 1H), 4.26 (s, 2H), 3.76 (s, 4H), 3.52 (s, 2H), 3.27 (d, J = 14.9 Hz, 6H).

5.9.22. (*E*)-2-(2-(4-Hydroxy-3,5-dimethoxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10e)

This compound was obtained as white solid in 88% yield. Mp 223.5–224.6 °C. ESI-MS m/z: $[M+Na]^+$ 486.1, $[M-H]^-$ 462.2. ^{1}H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 8.74 (s, 1H), 7.98 (s, 1H), 6.92 (s, 2H), 4.19 (s, 2H), 3.80 (s, 6H), 3.72 (s, 4H), 3.46 (s, 2H), 3.23 (d, J = 20.2 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 166.81, 162.30, 158.45, 148.59(2C), 142.11, 137.34, 126.15, 104.36(2C), 102.62, 66.33(2C), 56.40(2C), 49.36, 49.22(2C), 47.04, 32.31. ESI-HRMS m/z: calcd for $C_{20}H_{25}N_5O_6S$ $[M+H]^+$: 464.1640; found 464.1595.

5.9.23. (*E*)-4-((2-(4-Morpholino-6,6-dioxido-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-2-yl)hydrazono)methyl)benzonitrile (10f)

This compound was obtained as yellow solid in 85% yield. Mp 276.6–281.3 °C. ESI-MS m/z: $[M+H]^+$ 413.2. ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 8.13 (s, 1H), 7.83 (dd, J = 21.0, 8.4 Hz, 4H),

4.22 (s, 2H), 3.77–3.70 (m, 4H), 3.52–3.46 (m, 2H), 3.26 (s, 4H), 3.22 (t, J = 6.8 Hz, 2H).

5.9.24. (*E*)-2-(2-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10g)

This compound was obtained as white solid in 87% yield. Mp 277.6–278.0 °C. ESI-MS m/z: $[M+H]^+$ 516.2, $[M-H]^-$ 514.3. ^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 7.95 (s, 1H), 7.39 (s, 2H), 7.23 (s, 1H), 4.17 (s, 2H), 3.69 (s, 4H), 3.44 (s, 2H), 3.24 (s, 4H), 3.15 (s, 2H), 1.37 (s, 18H). ^{13}C NMR (126 MHz, DMSO) δ 166.74, 162.29, 158.53, 155.49, 139.63(2C), 127.26, 126.68, 123.43(2C), 102.43, 66.33(2C), 49.38, 49.16(2C), 47.04, 35.01(2C), 32.18, 30.67(6C).

5.9.25. (*E*)-2-(2-(3-Bromo-4-hydroxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10h)

This compound was obtained as white solid in 70% yield. Mp 279.1–280.2 °C. ESI-MS m/z: $[M+H]^+$ 484.0, $[M-H]^-$ 482.1. ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.65 (s, 1H), 7.98 (s, 1H), 7.76 (s, 1H), 7.45 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 4.20 (s, 2H), 3.73 (s, 4H), 3.47 (s, 2H), 3.22 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 166.87, 162.36, 158.38, 155.27, 140.55, 130.93, 128.53, 127.52, 117.00, 110.24, 102.96, 66.33(2C), 49.32, 49.26(2C), 47.02, 32.37.

5.9.26. (*E*)-2-(2-(2,4-Dihydroxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10i)

This compound was obtained as pale yellow solid in 78% yield. Mp >300 °C. ESI-MS m/z: $[M-H]^-$ 418.1. ^{1}H NMR (400 MHz, DMSO) δ 12.03 (s, 1H), 11.22 (s, 1H), 9.79 (s, 1H), 8.11 (s, 1H), 7.12 (d, J = 8.3 Hz, 1H), 6.33–6.29 (m, 1H), 6.28 (s, 1H), 4.20 (s, 2H), 3.72 (s, 6H), 3.26 (s, 4H), 3.18 (d, J = 6.7 Hz, 2H).

5.9.27. (*E*)-2-(2-(2,5-Dimethoxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10j)

This compound was obtained as pale yellow solid in 82% yield. Mp 239.1–240.0 °C. ESI-MS m/z: $[M+Na]^+$ 470.1, $[M-H]^-$ 446.2. ^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.39 (s, 1H), 7.38 (s, 1H), 7.00 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 9.5 Hz, 1H), 4.20 (s, 2H), 3.78 (s, 3H), 3.74 (s, 4H), 3.73–3.70 (m, 3H), 3.47 (d, J = 6.5 Hz, 2H), 3.26 (s, 4H), 3.21 (d, J = 6.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.80, 162.33, 158.41, 153.70, 152.00, 136.87, 124.61, 116.23, 113.64, 109.82, 102.94, 66.32(2C), 56.67, 55.78, 49.36, 49.20(2C), 47.01, 32.34.

5.9.28. (*E*)-2-(2-(3,5-Dimethoxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10k)

This compound was obtained as white solid in 80% yield. Mp 260.1–261.5 °C. ESI-MS m/z: $[M+H]^+$ 448.2, $[M-H]^-$ 446.2. ^{1}H NMR (400 MHz, DMSO) δ 10.95 (s, 1H), 8.38 (s, 1H), 7.78 (d, J = 9.2 Hz, 1H), 6.63 (s, 2H), 4.22 (s, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.76 (s, 4H), 3.52–3.47 (m, 2H), 3.28–3.18 (m, 6H).

5.9.29. (*E*)-4-Morpholino-2-(2-(3,4,5-trimethoxybenzylidene)-hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10l)

This compound was obtained as pale yellow solid in 75% yield. Mp 260.6–261.2 °C. ESI-MS m/z: $[M+H]^+$ 478.2. ^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 8.01 (s, 1H), 6.95 (s, 2H), 4.21 (s, 2H), 3.82 (s, 6H), 3.72 (s, 4H), 3.68 (s, 3H), 3.48 (t, J = 6.3 Hz, 2H), 3.27 (s, 4H), 3.20 (t, J = 6.5 Hz, 2H).

5.9.30. (*E*)-4-Morpholino-2-(2-(2,3,4-trimethoxybenzylidene)-hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10m)

This compound was obtained as pale yellow solid in 69% yield. Mp 243.4–244.0 °C. ESI-MS m/z: $[M+Na]^+$ 500.1, $[M-H]^-$ 476.2. ^{1}H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 8.31 (s, 1H), 7.56 (d, J = 8.9 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 4.21 (s, 2H), 3.91–3.76 (m, 9H), 3.72 (s, 4H), 3.50–3.45 (m, 2H), 3.22 (s, 6H).

5.9.31. (*E*)-2-(2-(4-Methoxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10n)

This compound was obtained as white solid in 81% yield. Mp 282.9–283.1 °C. ESI-MS m/z: $[M+H]^+$ 418.8. ^{1}H NMR (400 MHz, DMSO) δ 10.94 (s, 1H), 8.05 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 4.20 (s, 2H), 3.79 (s, 3H), 3.73 (s, 4H), 3.47 (s, 2H), 3.23 (s, 4H), 3.20 (s, 2H).

5.9.32. (*E*)-2-(2-(2,4-Dimethoxybenzylidene)hydrazinyl)-4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10o)

This compound was obtained as pale yellow solid in 77% yield. Mp 256.3–257.1 °C. ESI-MS m/z: $[M+H]^+$ 448.2. ^{1}H NMR (400 MHz, DMSO) δ 10.92 (s, 1H), 8.34 (s, 1H), 7.74 (d, J = 9.0 Hz, 1H), 6.60 (s, 2H), 4.19 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.73 (s, 4H), 3.46 (d, J = 6.4 Hz, 2H), 3.22 (s, 4H), 3.19 (s, 2H).

5.9.33. (*E*)-4-Morpholino-2-(2-(2-nitrobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10p)

This compound was obtained as pale yellow solid in 84% yield. Mp 276.3–276.9 °C. ESI-MS m/z: $[M+H]^+$ 433.1. ^{1}H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 8.49 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.76 (s, 1H), 7.58 (s, 1H), 4.22 (s, 2H), 3.73 (s, 4H), 3.48 (s, 2H), 3.27 (s, 4H), 3.22 (t, J = 6.4 Hz, 2H).

5.9.34. (*E*)-4-Morpholino-2-(2-(4-nitrobenzylidene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine 6,6-dioxide (10q)

This compound was obtained as orange-yellow solid in 77% yield. Mp 281.1–282.2 °C. ESI-MS m/z: $[M-H]^-$ 431.1. ^{1}H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 8.26 (d, J = 8.7 Hz, 2H), 8.19 (s, 1H), 7.88 (d, J = 8.6 Hz, 2H), 4.23 (s, 2H), 3.74 (s, 4H), 3.49 (s, 2H), 3.30–3.21 (m, 6H).

5.10. mTOR kinase assay[9]

The mTOR kinase activity of all the compounds (**7a–q**, **10a–q**) was determined using LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay following the manufacturer's instructions, with compounds mTOR inhibitors BMCL-200908069-1 and PI103 as positive controls. 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 $MnCl_2$, 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-anti-phospho-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.

5.11. PI3Kα kinase assay[10]

The selected compounds (**7b**, **7e**, **7h**, **10b** and **10e**) are tested for their activities against PI3Kα using a Kinase-Glo® Luminescent Kinase Assay, with PI103 as positive control. The kinase reaction is done in 384-well black plate. Each well is loaded with 50 μL of test items (in 90% DMSO) and 5 μL reaction buffer containing 10 μg/mL PI substrate (l-α-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 μL of 1 μM ATP prepared in the reaction buffer and is incubated for 60 min for p110α. It is terminated by the addition of 10 μL Kinase-Glo buffer. The plates are then read in a Synergy 2 reader for luminescence detection. All of the compounds were tested two times.

5.12. Cytotoxicity assay in vitro

The cytotoxic activities of five selected compounds (**7b**, **7e**, **7h**, **10b** and **10e**) were evaluated with H460, PC-3 cell lines by the standard MTT assay in vitro, with compounds mTOR inhibitors BMCL-200908069-1 and PI103 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 °C 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 μg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) 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.

5.13. Docking studies

For docking purposes, the three-dimensional structure of the mTOR (PDB code: 4JT6) were obtained from RCSB Protein Data Bank.[11] Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. The protonated state of several important residues, such as GLY2238, VAL2240 and TYR2225, 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 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.

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