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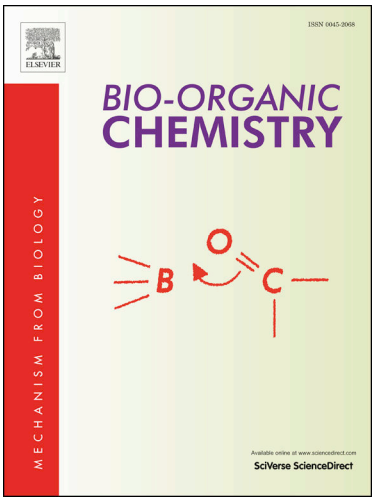

Design, synthesis of 1,3-dimethylpyrimidine-2,4-diones as potent and selective aldehyde dehydrogenase 1A1 (ALDH1A1) inhibitors with glucose consumption improving activity

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Design, synthesis of 1,3-dimethylpyrimidine-2,4-diones as potent and selective aldehyde dehydrogenase 1A1 (ALDH1A1) inhibitors with glucose consumption improving activity

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Abstract: ALDH1A1, one of 19 $NAD(P)^+$-dependent aldehyde dehydrogenases, participates in multiple metabolic pathways and has been indicated to play an important role in obesity and diabetes. In this study, a series of 1,3-dimethylpyrimidine-2,4-diones were designed, synthesized and evaluated as novel selective aldehyde dehydrogenase 1A1 inhibitors. Among them, compounds **46**, **50**, **53**, **56** and **57** exhibited excellent inhibitory activity against ALDH1A1 with IC_{50} values in the low nanomolar range and high selectivity over ALDH1A2, ALDH1A3, ALDH2 and ALDH3A1. Furthermore, *in vitro* study demonstrated that compound **57** effectively improved glucose consumption in HepG2 cells compared to compound **1** (CM026).

Key words: ALDH1A1; enzyme inhibition; selective; synthesis; glucose consumption improvement

1. Introduction

Human aldehyde dehydrogenases (ALDHs) comprise a superfamily of at least 19 isozymes, most of which catalyze the NAD $(P)^+$- dependent oxidation of both endogenous and exogenous aldehydes to their respective carboxylic acid derivatives [1]. ALDHs act as a key regulator in multiple metabolic pathways and have been indicated to possess important physiological and toxicological functions in several areas such as cancers, metabolic disorders and other diseases [2,3]. Mutations in ALDHs genes can lead to aberrant aldehyde oxidation and have been proved to be associated with the occurrence and development of a series of human diseases, including Parkinson's disease, alcoholism and diabetes [4-7].

Up-regulation of ALDH1A1, also known as retinaldehyde dehydrogenase 1(RALDH1), has been implicated in obesity and diabetes [7-9]. Several studies have demonstrated that ALDH1A1 knockout mice were able to resist diet-induced obesity as well as insulin resistance [10,11]. In addition, treatment with exogenous retinaldehyde can reduce hepatic gluconeogenesis and lipid accumulation in *ob*/*ob* mice [11,12]. These results indicate that ALDH1A1 plays a crucial role in obesity and adipogenesis. Previous researches in our lab have revealed that ALDH1A1 inhibitor citral is capable of significantly improving glucose metabolism through inhibiting ALDH1A1 activity [13]. These findings suggest that inhibition of ALDH1A1 enzymatic activity may provide a new therapeutic option for related diseases.

Several small molecule ALDH1A1 inhibitors identified by high throughput screening (HTS) have been reported (**Fig. 1**). Previous studies by Hurley and colleagues demonstrated that theophylline-based **1** (CM026) and substituted tricyclic pyrimidinone **2** (CM37) displayed effective ALDH1A1 inhibitory activity (0.8 and 0.02 μM for 1 and 2, respectively) and showed excellent selectivity against other

ALDH isozymes, such as ALDH2 and ALDH3A1 [14]. Quinoline-based compound **3** (NCT-505) disclosed by Yang *et al.* exhibited potent and highly selective ALDH1A1 inhibition (1A1 IC_{50} = 0.007 μM, 1A2, 1A3, ALDH2, ALDH3A1 IC_{50} values > 1 μM) and displayed efficacious cellular activities in MIA PaCa-2 cells, which preferentially express ALDH1A1 [15].

Most recently, we reported a series of coumarin-based derivatives (e.g., **4a** and **4b**) with potent inhibitory activity to ALDH1A1 and high selectivity against other ALDH isozymes. Among them, **4a** was the most potent compound with an IC_{50} value of 1.96 μM. Furthermore, Compound **4b** can dramatically alleviate palmitic acid-induced impairment of glucose consumption in HepG2 cells [16].

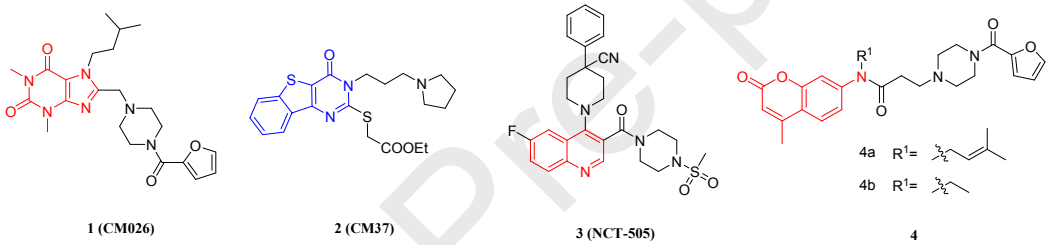


Fig. 1. Representative reported ALDH1A1 inhibitors.

Compound **1** has a unique bicyclic core attached with two adjacent arms. By analyzing the crystal structure of ALDH1A1 complexed with compound **1** (PDB code 4WPN), we found that the xanthine ring interacts with His293, Cys302, and Gly458 through crucial hydrogen bond respectively (**Fig. 2**). However, N^7 atom in the imidazole ring exhibit no interaction with any amino residues of ALDH1A1. It has been considered that theophylline was inactive to inhibit ALDH1A1 at concentrations up to 250 μM, suggesting that two adjacent arms attached to the theophylline perform an important role in ALDH1A1 inhibitory activity [14].

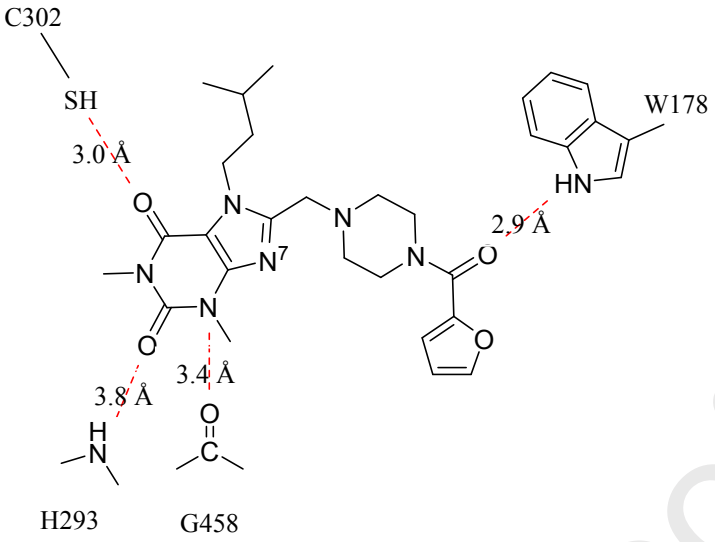


Fig. 2. Structure analysis of human ALDH1A1 with compound **1** (CM026) (PDB code 4WPN, the key hydrogen bonds, illustrated with red dashed lines)

Thus a series of 1,3-dimethylpyrimidine-2,4-diones were designed through opening the imidazole ring of compound **1** (**Fig. 3**), two substituted groups were introduced to N^5 to mimic the two adjacent arms of compound **1**.

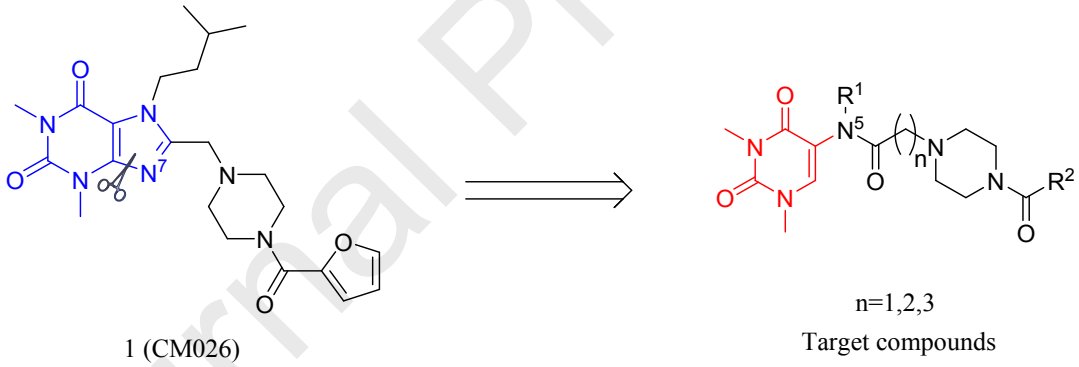


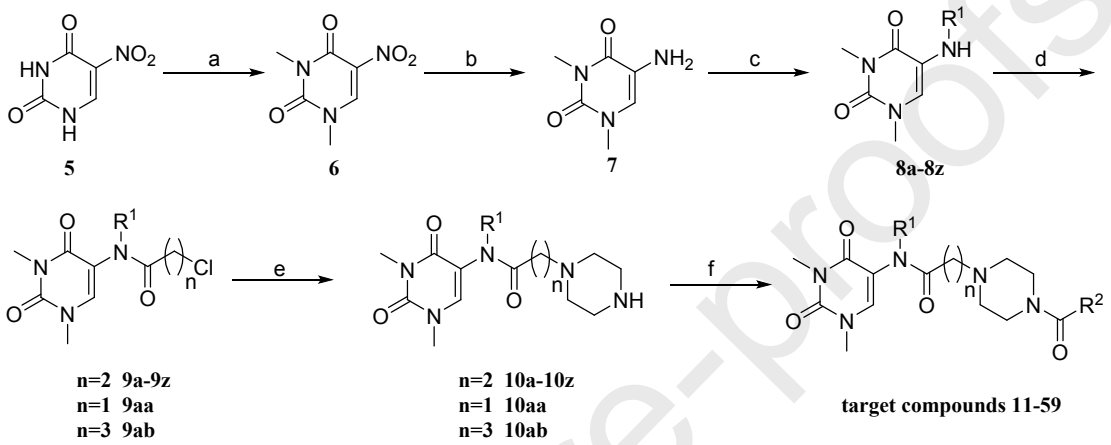
Fig. 3. Design of target compounds

2. Results and discussion

2.1. Chemistry

The designed compounds were synthesized following the synthetic route shown in **Scheme 1**. The starting material 5-nitropyrimidine-2,4(1*H*,3*H*)-dione (**5**) was allowed to react with iodomethane and sodium hydride to give intermediate **6**.

Reduction of the nitro group of **6** gave the amine **7**. Reaction of **7** with various halides or aldehydes and sodium cyanoborohydride led to derivatives **8a-8z**. **8a-8z** reacted with different acyl chlorides to afford intermediates **9a-9ab** and then **9a-9ab** reacted with piperazine to generate compounds **10a-10ab**. Condensation of **10a-10ab** with different commercial acids afforded target compounds **11-59** with good yields.



Scheme 1. Reagents and conditions: (a) CH_3I, NaH, DMF, 0-25℃, 20 h; (b) H_2, 10% Pd/C, MeOH, 25℃, 2 h; (c) For **8a**, **8l-8z**, R^1-X (X=Br or I), K_2CO_3, DMF, 25℃, 2-3 h; For **8b-8f**, R^1-X (X=Br or I), K_2CO_3, DMF, 60℃, 5-10 h; For **8g-8k**, R^1CHO, CH_3COOH, $NaBH_3CN$, DCM, 25℃, 3-7 h; (d) For **9a-9z**, 3-Chloropropionyl chloride, Et_3N, DCM, 25℃, 2-3 h; For **9aa**, Chloroacetyl chloride, Et_3N, DCM, 25℃, 2-3 h; For **9ab**, 4-Chlorobutyryl chloride, Et_3N, DCM, 25℃, 2-3 h; (e) piperazine, K_2CO_3, KI, CH_3CN, 25℃, overnight; (f) R^2COOH, EDCI, Et_3N, DCM, 25℃, overnight.

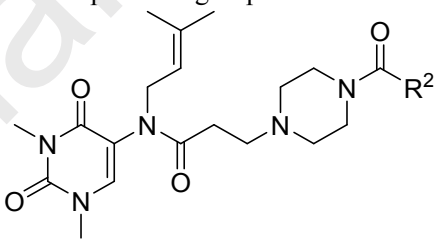
2.2. Biological evaluation

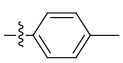
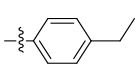
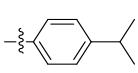
2.2.1. Inhibitory activity against ALDH1A1

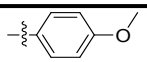
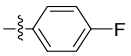
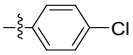
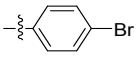
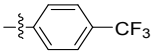
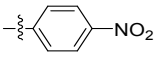
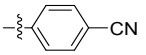
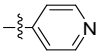
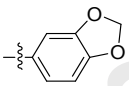
The inhibitory activities of the synthesized 1,3-dimethylpyrimidine-2,4-diones were initially screened at a relatively high single concentration (10 μM) to identify the most potent antagonist. Compound **1** (CM026), which was synthesized according to the literature, was used as a control [3]. The inhibition rate of compound **1** was 75.22% at the concentration of 10 μM. The influence of the terminal substituted group (R^2) on inhibitory activity for ALDH1A1 was initially explored as shown in **Table 1**. In these

compounds, the R^1 group was fixed as isopentenyl and the length of linker between the N^5 atom attached to the core structure 1,3-dimethylpyrimidine-2,4-diones and piperazine was kept as three-carbon atoms, same as compound **4a**. Compound **11** with unsubstituted phenyl and compound **12** with *para*-methyl phenyl substitution showed decreased activity compared to compound **1**. Compounds **13-15** containing an electron-donating group at the *para* position of phenyl showed maintained inhibitory activity (68.27%, 77.15 % and 60.51 % respectively at 10 μM). However, decreased activities were observed when electron-withdrawing substituent attached to terminal phenyl group (compounds **16-21)**. Other compounds, including five membered aromatic heterocyclic (**22**), nitrogen-containing aromatic heterocycles (**23** and **24**) and aliphatic ring (**26**) substitution exhibited poor inhibition to ALDH1A1. All results of inhibitory activity against ALDH1A1 demonstrated that R^2 group was critical to inhibitory activity and phenyl bearing an electron-rich substituent was the optimal group. Among this series of compounds, the preferred R^2 group was 4-isopropylphenyl.

Table 1. Structure-activity relationships for R^2 group.



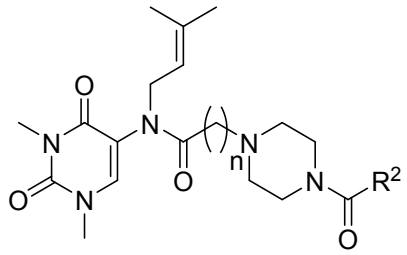
Compounds	R^2	hALDH1A1 % inhibition at 10 μM[a]
1 (CM026)		75.22 ± 5.28
11		39.23 ± 2.97
12		47.31 ± 3.69
13		68.27 ± 5.41
14		77.15 ± 7.02

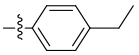
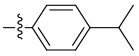
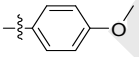
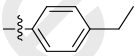
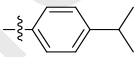
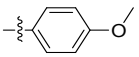
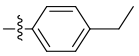
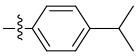
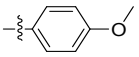
15		60.51 ± 5.78
16		31.19 ± 3.18
17		37.43 ± 3.20
18		24.42 ± 2.56
19		3.86 ± 0.43
20		28.33 ± 2.12
21		24.52 ± 2.09
22		9.44 ± 1.01
23		30.95 ± 2.66
24		36.65 ± 3.03
25		35.93 ± 3.32
26		0.05 ± 0.18

[a] Values are the mean ± SD of three independent experiments (n = 3)

To investigate the effect of the length of linker between N^5 atom and piperazine, compounds **27-34** were then designed and synthesized. In these compounds, the R^1 group was kept as isopentenyl and R^2 group was 4-methylphenyl, 4-ethylphenyl, 4-isopropylphenyl and 4-methoxyphenyl, respectively. It was observed that compounds **27-34**, with the linker between N^5 and piperazine of 2 or 4 atoms (n=1 or 3), showed significantly decreased inhibitory activities compared to compounds **12-15**. This indicates that the length of the linker exerted a crucial influence on the inhibitory activity against ALDH1A1 and the optimal length is three carbon atoms (n=2 in **Table 2**).

Table 2. Structure-activity relationships for the length of linker between N^5 and piperazine.



Compounds	n	R^2	hALDH1A1 % inhibition at 10 μM[a]
1 (CM026)			75.22 ± 5.28
12	2		47.31 ± 3.69
13	2		68.27 ± 5.41
14	2		77.15 ± 7.02
15	2		60.51 ± 5.78
27	1		8.50 ± 0.92
28	1		16.79 ± 1.23
29	1		14.47 ± 1.18
30	1		29.37 ± 2.78
31	3		32.19 ± 2.56
32	3		27.23 ± 1.64
33	3		33.01 ± 2.82
34	3		13.10 ± 0.77

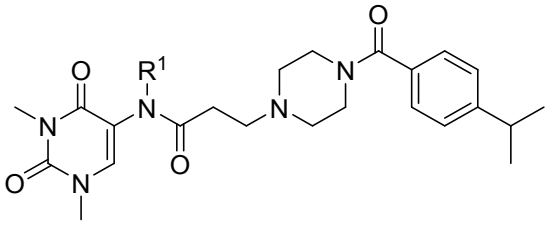
[a] Values are the mean ± SD of three independent experiments (n = 3)

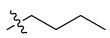
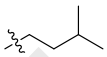
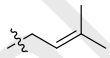
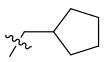
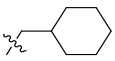
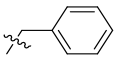
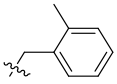
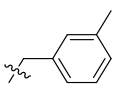
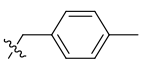
Then compounds **35-59** were designed and synthesized in order to explore the effect of R^1 group on enzyme inhibitory activity. In these compounds, the R^2 group was 4-isopropylphenyl, the length of linker between N^5 and piperazine was kept as three atoms and various substituted groups R^1 were introduced to replace the isopentenyl of compound **14**. As shown in **Table 3**, dramatically decreased inhibitory activities were observed for compounds **35-39** with R^1 group of an alkyl chain and the inhibitory activity showed increased tendency accordingly with the extension of the chain.

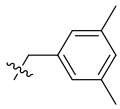
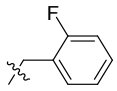
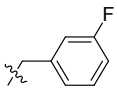
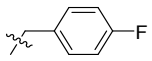
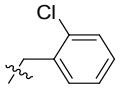
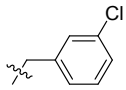
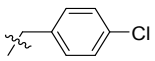
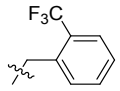
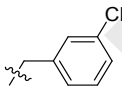
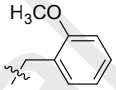
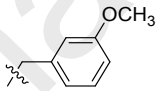
Compound **40** (R^1 = isopentyl) displayed comparable inhibitory activity to compound **14.** Compounds **41-43** displayed slightly decreased potency for which the isopentenyl was replaced by a small alicyclic group. Compound **44** (R^1 = cyclohexylmethyl) possessed a preferable inhibitory activity compared to compound **14.** In these four compounds, the inhibitory activity increased accordingly with the enlargement of the ring, indicating that bulky group at this portion was favorable. Compound **45** (R^1 = benzyl) showed outstanding potency and the data obtained from R^1 modifications suggested that aromatic group was beneficial for improvement of the inhibitory activity. Compounds **46-59** were thus designed and evaluated for their inhibitory activities. Introduction of a halo atom to the *para* position of phenyl group displayed slightly decreased inhibition (compounds **52** and **55** *vs* **45**), indicating that a substituted group on the *para* position was negative to inhibitory activity. Different from *para* substitutions, introduction a halo atom to the *ortho* and *meta* position exhibited more outstanding inhibitory activity. For example, compounds **53** and **54** which have a chloro atom on the *ortho* and *meta* position of the phenyl respectively showed more potent inhibitory activities compared to *para* position. The IC_{50} values for compounds **53** and **54** were 0.126 and 0.385 μM respectively, indicating that compared to *meta* position of phenyl, introducing a substituted group to *ortho* position could be more beneficial to improve inhibitory activity. Similarly, introduction of other hydrophobic groups, such as methyl, methoxyl and trifluoromethyl groups (compounds **46-48** and **56-59**) to different positions of phenyl ring showed the same trend. These results illustrated that the position of substituents had significant influence on inhibitory activity. Results of inhibitory activities against ALDH1A1 obtained from compounds **35-59** showed that the preferred R^1 groups are 2-methylphenyl (**46**, 0.149 μM), 2-fluorophenyl (**50**, 0.429

μM), 2-chlorophenyl (**53**, 0.126 μM), 3-chlorophenyl (**54**, 0.385 μM), 2-trifluoromethylphenyl (**56**, 0.059 μM), and 3-trifluoromethylphenyl (**57**, 0.379 μM).

Table 3. Structure-activity relationships for R^1 group.



Compounds	R^1	hALDH1A1	
		% inhibition at 10 μM [a]	IC_{50} ± SD [μM] [a]
1 (CM026)		75.22 ± 5.28	1.21 ± 0.18
35		27.18 ± 2.09	ND
36		31.75 ± 3.11	ND
37		20.00 ± 1.66	ND
38		53.33 ± 4.57	ND
39		38.67 ± 3.34	ND
40		74.49 ± 6.76	ND
14		77.15 ± 7.02	ND
41		41.33 ± 3.35	ND
42		57.33 ± 4.89	ND
43		69.33 ± 6.69	ND
44		82.67 ± 7.34	ND
45		86.41 ± 8.12	1.135 ± 0.26
46		98.67 ± 8.91	0.149 ± 0.11
47		95.15 ± 9.36	0.715 ± 0.19
48		89.32 ± 9.01	1.144 ± 0.31

49		74.03 ± 7.59	ND
50		90.04 ± 8.98	0.429 ± 0.08
51		93.23 ± 9.01	1.075 ± 0.35
52		74.03 ± 6.86	ND
53		100 ± 9.71	0.126 ± 0.08
54		100 ± 8.98	0.385 ± 0.10
55		80.89 ± 8.12	ND
56		100 ± 10.09	0.059 ± 0.02
57		100 ± 9.57	0.379 ± 0.07
58		94.94 ± 9.22	0.872 ± 0.23
59		93.50 ± 9.24	0.966 ± 0.13

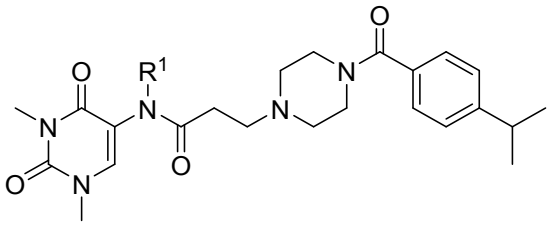
[a] Values are the mean ± SD of three independent experiments (n = 3)

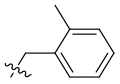
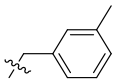
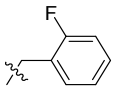
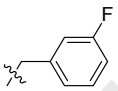
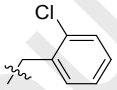
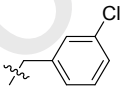
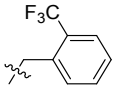
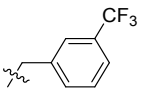
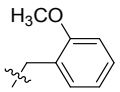
2.2.2. Selectivity evaluation of selected analogs against other ALDH isozymes.

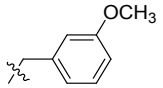
Structural research demonstrated that ALDH1A1 shares more than 70 % sequence similarity with both ALDH1A2 and ALDH1A3, nearly 70 % with the mitochondrial ALDH2 enzyme and less than 50 % with ALDH3A1 [17]. In order to verify the enzyme selectivity of the compounds we designed, compounds with excellent ALDH1A1 inhibitory activity (**46**, **47**, **50**, **51**, **53**, **54 and 56-59**) were selected to screen against a panel of enzymes including ALDH1A subfamily (ALDH1A2, ALDH1A3) and ALDH

isozymes (ALDH2, ALDH3A1). As shown in **Table 4**, all tested compounds exhibited almost no inhibition and showed excellent selectivity toward other ALDH isozymes.

Table 4. Selectivity against other ALDH isozymes.



Compounds	R^1	hALDH1A1 IC_{50} [μM] [a]	% inhibition at 10 μM [a]			
			ALDH1A2	ALDH1A3	ALDH2	ALDH3A1
1 (CM026)		1.21 ± 0.18	0.11 ± 0.07	0.09 ± 0.03	0.13 ± 0.04	0.97 ± 0.14
46		0.149 ± 0.11	0.13 ± 0.05	0.15 ± 0.01	0.05 ± 0.01	5.26 ± 0.92
47		0.715 ± 0.19	0.90 ± 0.17	0.34 ± 0.05	0.09 ± 0.01	9.09 ± 1.75
50		0.429 ± 0.08	0.25 ± 0.08	0.35 ± 0.02	4.79 ± 0.95	4.07 ± 0.72
51		1.075 ± 0.35	0.38 ± 0.07	0.45 ± 0.04	3.78 ± 0.77	2.63 ± 0.48
53		0.126 ± 0.08	0.82 ± 0.08	0.32 ± 0.04	2.77 ± 0.32	6.05 ± 0.88
54		0.385 ± 0.10	0.76 ± 0.09	0.21 ± 0.02	8.06 ± 2.12	1.77 ± 0.28
56		0.059 ± 0.02	1.57 ± 0.31	0.39 ± 0.03	0.06 ± 0.01	5.74 ± 1.18
57		0.379 ± 0.07	2.68 ± 0.65	6.25 ± 1.01	0.08 ± 0.03	3.11 ± 0.59
58		0.872 ± 0.23	1.65 ± 0.26	0.68 ± 0.08	0.19 ± 0.05	0.19 ± 0.03

59		0.966 ± 0.13	1.87 ± 0.17	0.48 ± 0.12	0.21 ± 0.06	0.32 ± 0.07

[a] Values are the mean ± SD of three independent experiments (n=3).

2.2.3. Cell viability assay of representative ALDH1A1 inhibitors.

In order to evaluate the cytotoxicity of our synthesized compounds, the viability of HepG2 cells that treated with representative ALDH1A1 inhibitors (**46**, **50**, **53**, **56** and **57**) at different concentrations was determined. As shown in Figure 4, all tested compounds showed negligible cytotoxic effect on HepG2 cells at concentrations of 0.1 μM, 1 μM and 10 μM, and the morphology of HepG2 cells was not affected, which indicated that these inhibitors have low cytotoxicity to HepG2 cells.

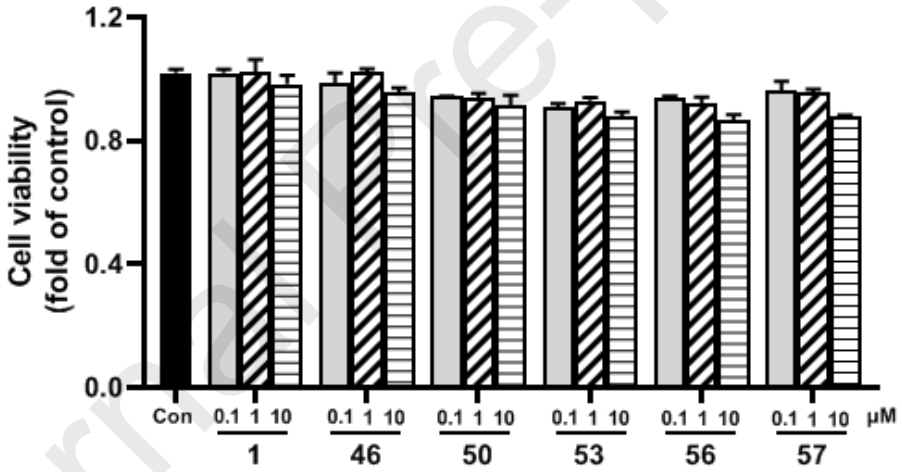


Fig. 4. Cell viability assay of compound **1** and representative compounds. Data were expressed in mean ± S.D. (n = 4).

2.2.4. Improvement of glucose consumption in HepG2 cells.

To demonstrate the potential utility in the improvement of glucose consumption, these selective ALDH1A1 inhibitors (**46**, **50**, **53**, **56** and **57**) and positive control **Metformin** were evaluated in HepG2 cells according to the reported method [18]. Results showed that compared to the blank control, compound **1** exhibited no effect on glucose metabolism in HepG2 cells at concentrations of 1 μM and 10 μM. Among all

tested five compounds, **57** displayed about 20% improvement on glucose metabolism in HepG2 cells at 1 μM. All these five compounds can significantly improve glucose metabolism in HepG2 cells at 10 μM. The glucose consumption of compounds **46**, **50**, **53**, **56** and **57** increased by 10%, 10%, 15%, 20% and 30% respectively (**Fig. 5**). Moreover, compound **57** at 10 μM produced equivalent glucose consumption improvement in HepG2 cells to positive control Metformin (Met) at 1 mM.

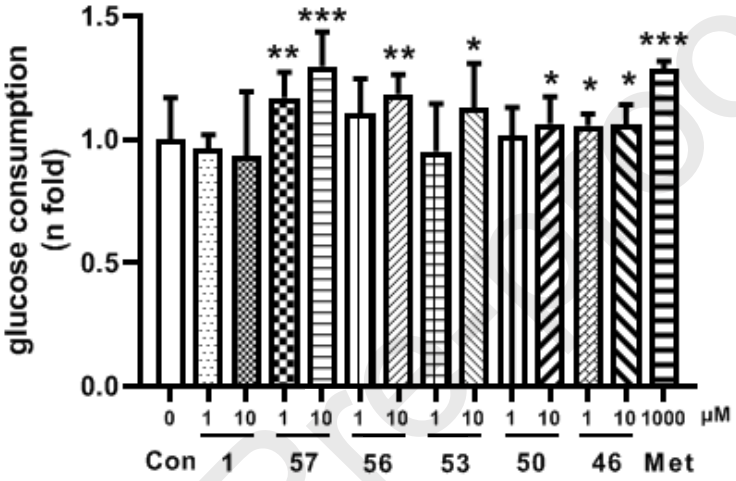


Fig. 5. Effects of compound **1** and representative compounds on glucose consumption in HepG2 cells. Data were expressed in mean ± S.D. (n = 4). *p < 0.05, **p < 0.01, ***p < 0.001 versus untreated control cells (Con).

2.2.5 Metabolic stability assay in human liver microsomal of optimized compounds.

The potent compounds **53**, **56** and **57** were then chosen to evaluate their metabolic stability in human microsomes. As shown in **Table 5**, the tested three compounds showed microsomal stability with $T_{1/2}$ values of 3, 10 and 12 min respectively. Thus, improving the metabolic stability of these compounds through further structural modifications has become our primary object next step.

Table 5. Metabolic parameters in human liver microsomal.

Parameters	**53**	**56**	**57**
k(min^{-1})	0.32	0.072	0.059

$t_{1/2}$(min)	3	10	12
P_m(mg/mL)	0.25	0.25	0.5
C_{lint}(μL/min/mg)	1.28	0.288	0.118

3. Conclusion

In summary, a series of 1,3-dimethylpyrimidine-2,4-diones were designed, synthesized and evaluated as potent and selective ALDH1A1 inhibitors. Several of them presented potent inhibitory activities against ALDH1A1 with IC_{50} values in the low nanomolar range and a high degree of selectivity over other ALDH isozymes (ALDH1A2, ALDH1A3, ALDH2 and ALDH3A1). Compounds **46**, **50**, **53**, **56** and **57** exhibited more potent activity in enzymatic assays comparing to compound **1**. These compounds also effectively improved glucose consumption in HepG2 cells and the compound **57** was the most potent**.** Although these compounds exhibit excellent activities *in vitro*, their metabolic stability is poor. So, further work has been focused on the structural optimization of these compounds to improve their metabolic stability and this work is proceeding.

4. Experimental section

4.1. Chemistry

Chemical reagents and solvents were obtained from commercial sources and used without further purification. All reactions were monitored by thin-layer chromatography (TLC) on silica gel plates and visualized under UV light. Silica gel flash chromatography was performed using silica gel (300-400 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 300 MHz or 400 MHz spectrometer using deuterated solvents with tetramethylsilane as an internal standard. Chemical shifts were

reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t) and multiplet (m). The purity of the synthesized compounds were measured by analytical high performance liquid chromatography (HPLC, Agilent) using the C_{18} reverse phase column. High-resolution mass spectroscopic (HRMS) measurements were performed on an Agilent QTOF 6520 mass spectrometer with electron spray ionization (ESI) as the ion source.

*4.1.1. 1,3-Dimethyl-5-nitropyrimidine-2,4(1H,3H)-dione (**6**).*

To a solution of 5-nitropyrimidine-2,4(1*H*,3*H*)-dione (1.0 g, 6.37 mmol) in DMF (10 ml) was added NaH (0.61 g, 15.28 mmol) and stirred at 0 ℃ for 2 h. The mixture was added CH_3I (2.26 g, 15.91 mmol) and stirred at room temperature for 20 h. After cooling to 0 ℃, the mixture was added ice water (30 ml) and then stirred for 30 min. The solid was collected by filtration and washed successively with H_2O (2× 10 mL) to give a pale yellow product (0.95 g, 81 % yield). Mp 155-157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.28 (s, 1H), 3.40 (s, 3H), 3.13 (s, 3H). ESI-MS *m/z*: 186.1 $[M+H]^+$.

*4.1.2 5-Amino-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**7**).*

To a mixture of 1,3-dimethyl-5-nitropyrimidine-2,4(1*H*,3*H*)-dione **6** (1 g, 5.4 mmol) in MeOH (20 mL) was added 10% Pd/C (100 mg). The air was removed and refilled with H_2 gas (3 times). The mixture was stirred at room temperature for 2 h under H_2 gas. The solvent was filtered through Celite and eluted with CH_2Cl_2. The filtrate was concentrated in vacuo to give 5-amino-1,3-dimethylpyrimidine-2,4(1H,3H)-dione as a white solid which was used without purification (0.75 g, 90 % yield). ^{1}H NMR (300 MHz, DMSO) δ 6.84 (s, 1H), 4.16 (s, 2H), 3.21 (d, J = 10.6 Hz, 6H). ESI-MS *m/z*: 156.2 $[M+H]^+$.

*4.1.3 General procedure for preparation of compounds (**8a, 8l-8z**)*.

To a solution of 5-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione **7** (1 g, 6.45 mmol) in DMF (10 mL) was added potassium carbonate (1.78 g, 12.89 mmol) and stirred at 0℃ for 0.5 h. Then different halides (7.09 mmol) was added sequentially and stirred at room temperature for 1.5 h. The resulted solution was poured into water (50 mL) and extracted with EtOAc (45 mL × 2). The combined organic layer was dried (Na_2SO4) and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **8a**, **8l-8z** respectively.

*4.1.3.1. 5-((3-Methylbut-2-en-1-yl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8a**)*. A yellow oil. Yield 58 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.12 (s, 1H), 5.27 (t, J = 6.6 Hz, 1H), 3.87 (s, 1H), 3.51 - 3.42 (m, 2H), 3.38 (d, J = 4.9 Hz, 6H), 1.74 (s, 3H), 1.68 (s, 3H). ESI-MS *m/z*: 224.2 $[M+H]^+$.

*4.1.3.2. 5-(Benzylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8l**)*. A pale yellow solid. Yield 61 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 - 7.27 (m, 5H), 6.07 (s, 1H), 4.16 (s, 2H), 3.39 (s, 3H), 3.29 (s, 3H). ESI-MS *m/z*: 246.1 $[M+H]^+$.

*4.1.3.3. 5-((2-Methylbenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8m**)*. A pale yellow solid. Yield 65 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 - 7.24 (m, 1H), 7.23 - 7.13 (m, 3H), 6.11 (s, 1H), 4.27 (s, 1H), 4.07 (s, 2H), 3.39 (d, J = 1.6 Hz, 3H), 3.33 (d, J = 1.6 Hz, 3H), 2.34 (s, 3H). ESI-MS *m/z*: 260.3 $[M+H]^+$.

*4.1.3.4. 5-((3-Methylbenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8n**)*. A pale yellow solid. Yield 60 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, J = 7.4 Hz, 1H), 7.16 - 7.08 (m, 3H), 6.07 (s, 1H), 4.42 (s, 1H), 4.11 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H), 2.35 (s, 3H). ESI-MS *m/z*: 260.3 $[M+H]^+$.

*4.1.3.5. 5-((4-Methylbenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8o**)*. A pale yellow solid. Yield 62 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 7.8 Hz, 2H), 6.07 (s, 1H), 4.41 (s, 1H), 4.09 (s, 2H), 3.38 (s, 3H), 3.28 (s, 3H), 2.33 (s, 3H). ESI-MS *m/z*: 260.3 $[M+H]^+$.

*4.1.3.6. 5-((3,5-Dimethylbenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8p**)*. A pale yellow solid. Yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 - 6.87 (m, 3H), 6.08 (s, 1H), 4.39 (s, 1H), 4.05 (s, 2H), 3.38 (s, 3H), 3.29 (s, 3H), 2.29 (s, 6H). ESI-MS *m/z*: 274.3 $[M+H]^+$.

*4.1.3.7. 5-((2-Fluorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8q**)*. A pink solid. Yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.20 (m, 2H), 7.18 - 6.99 (m, 2H), 6.16 (s, 1H), 4.45 (s, 1H), 4.21 (s, 2H), 3.38 (s, 3H), 3.31 (s, 3H). ESI-MS *m/z*: 264.2 $[M+H]^+$.

*4.1.3.8. 5-((3-Fluorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8r**)*. A pink solid. Yield 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.24 (m, 1H), 7.15 - 6.90 (m, 3H), 6.03 (s, 1H), 4.55 (s, 1H), 4.18 (s, 2H), 3.39 (s, 3H), 3.29 (s, 3H). ESI-MS *m/z*: 264.2 $[M+H]^+$.

*4.1.3.9. 5-((4-Fluorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8s**)*. A pink solid. Yield 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 - 7.24 (m, 2H), 7.10 - 6.97 (m, 2H), 6.05 (s, 1H), 4.41 (s, 1H), 4.12 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H). ESI-MS *m/z*: 264.2 $[M+H]^+$.

*4.1.3.10. 5-((2-Chlorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8t**)*. A yellow solid. Yield 52%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.34 (m, 2H), 7.27 - 7.22

(m, 2H), 6.08 (s, 1H), 4.54 (s, 1H), 4.26 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H). ESI-MS *m/z*: 280.2 $[M+H]^+$.

*4.1.3.11. 5-((3-Chlorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8u**).* A yellow solid. Yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.30 - 7.24 (m, 2H), 7.21 (d, J = 6.2 Hz, 1H), 6.02 (s, 1H), 4.50 (s, 1H), 4.16 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H). ESI-MS *m/z*: 280.2 $[M+H]^+$.

*4.1.3.12. 5-((4-Chlorobenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8v**).* A yellow solid. Yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (q, J = 8.5 Hz, 4H), 6.02 (s, 1H), 4.47 (s, 1H), 4.14 (s, 2H), 3.40 (s, 3H), 3.29 (s, 3H). ESI-MS *m/z*: 280.2 $[M+H]^+$.

*4.1.3.13. 5-((2-(Trifluoromethyl)benzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8w**).* A yellow solid. Yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 - 7.41 (m, 3H), 7.35 - 7.27 (m, 1H), 5.95 (s, 1H), 4.62 (s, 1H), 4.31 (s, 2H), 3.30 (s, 3H), 3.19 (s, 3H). ESI-MS *m/z*: 314.2 $[M+H]^+$.

*4.1.3.14. 5-((3-(Trifluoromethyl)benzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8x**).* A yellow solid. Yield 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.58 - 7.44 (m, 3H), 6.05 (s, 1H), 4.56 (s, 1H), 4.24 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H). ESI-MS *m/z*: 314.2 $[M+H]^+$.

*4.1.3.15. 5-((2-Methoxybenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8y**).* A white solid. Yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 - 7.20 (m, 2H), 6.97 - 6.90 (m, 2H), 6.89 - 6.86 (m, 1H), 6.16 (s, 1H), 4.44 (s, 1H), 4.14 (s, 2H), 3.85 (s, 3H), 3.38 (s, 3H), 3.30 (s, 3H). ESI-MS *m/z*: 276.1 $[M+H]^+$.

*4.1.3.16. 5-((3-Methoxybenzyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8z**).* A white solid. Yield 61%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 - 7.22 (m, 1H), 6.94 - 6.77 (m, 3H), 6.06 (s, 1H), 4.49 (s, 1H), 4.14 (s, 2H), 3.80 (s, 3H), 3.39 (s, 3H), 3.29 (s, 3H). ESI-MS *m/z*: 276.1 $[M+H]^+$.

*4.1.4. General procedure for preparation of compounds (**8b-8f**).*

To a solution of 5-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione **7** (1 g, 6.45 mmol) in DMF (10 mL) was added potassium carbonate (1.78 g, 12.89 mmol) and stirred at 0℃ for 0.5 h. Then different halides (7.09 mmol) was added sequentially and stirred at 60 °C for 5-10 h until the reaction was completed. After cooling to room temperature, the resulted solution was poured into water (50 mL) and extracted with EtOAc (45 mL × 2). The combined organic layer was dried (Na_2SO_4) and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **8b-8f** respectively.

*4.1.4.1. 5-(Ethylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8b**).* A yellow oil. Yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (s, 1H), 3.89 (s, 1H), 3.34 (s, 3H), 3.30 (s, 3H), 2.92 - 2.83 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H). ESI-MS *m/z*: 184.2 $[M+H]^+$.

*4.1.4.2. 5-(Propylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8c**).* A yellow oil. Yield 50%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (s, 1H), 3.85 (s, 1H), 3.35 (d, J = 4.8 Hz, 6H), 2.87 - 2.76 (m, 2H), 1.71 - 1.56 (m, 2H), 0.97 (t, J = 7.5 Hz, 3H). ESI-MS *m/z*: 198.3 $[M+H]^+$.

*4.1.4.3. 5-(Isopropylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8d**).* A yellow oil. Yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (s, 1H), 3.32 (d, J = 1.6 Hz, 6H), 3.23 - 3.12 (m, 1H), 1.12 (d, J = 6.2 Hz, 6H). ESI-MS *m/z*: 198.3 $[M+H]^+$.

*4.1.4.4. 5-(Butylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8e**).* A white oil. Yield 48%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (s, 1H), 3.33 (d, J = 3.1 Hz, 6H), 2.88 - 2.76 (m, 2H), 1.64 - 1.50 (m, 2H), 1.45 - 1.33 (m, 2H), 0.90 (t, J = 7.5 Hz, 3H). ESI-MS *m/z*: 212.2 $[M+H]^+$.

*4.1.4.5. 5-(Isobutylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8f**).* A white oil. Yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (s, 1H), 3.94 (s, 1H), 3.39 (s, 3H), 3.37 (s, 3H), 2.72 - 2.65 (m, 2H), 1.95 - 1.83 (m, 1H), 0.99 (s, 3H), 0.98 (s, 3H). ESI-MS *m/z*: 212.2 $[M+H]^+$.

*4.1.5. General procedure for preparation of compounds (**8g-8k**).*

To a solution of 5-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione **7** (1 g, 6.45 mmol) in DCM (20 mL) was added acetic acid (0.04 g, 0.65 mmol) and different aliphatic aldehydes (7.73 mmol). The mixture was stirred at room temperature for 1 h. Then sodium cyanoborohydride (0.81 g, 12.89 mmol) was added sequentially and stirred at room temperature for 3-7 h until the reaction was completed. The reaction was quenched with a saturated aqueous solution (50 mL) and the mixture was extracted with DCM (50 mL × 3). The combined organic layer was dried over anhydrous sodium sulfate and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **8g**-**8k** respectively.

*4.1.5.1. 5-(Isopentylamino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8g**).* A yellow oil. Yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.04 (s, 1H), 3.73 (s, 1H), 3.29 - 3.19 (m, 6H), 2.81 - 2.70 (m, 2H), 1.66 - 1.51 (m, 1H), 1.46 - 1.33 (m, 2H), 0.81 (d, J = 6.5 Hz, 6H). ESI-MS *m/z*: 226.3 $[M+H]^+$.

*4.1.5.2. 5-((Cyclopropylmethyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8h**).* A yellow oil. Yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.06 (s, 1H), 3.30 (d, J = 6.1

Hz, 6H), 2.69 - 2.60 (m, 2H), 1.06 - 0.97 (m, 1H), 0.54 - 0.45 (m, 2H), 0.20 - 0.09 (m, 2H). ESI-MS *m/z*: 210.3 $[M+H]^+$.

*4.1.5.3. 5-((Cyclobutylmethyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8i**).* A yellow oil. Yield 47%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (s, 1H), 3.80 (s, 1H), 3.37 (s, 3H), 3.36 (s, 3H), 2.87 (d, J = 7.2 Hz, 2H), 2.63 - 2.52 (m, 1H), 2.15 - 2.06 (m, 2H), 1.98 - 1.82 (m, 2H), 1.76 - 1.66 (m, 2H). ESI-MS *m/z*: 224.3 $[M+H]^+$.

*4.1.5.4. 5-((Cyclopentylmethyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8j**).* A yellow oil. Yield 41%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (s, 1H), 3.89 (s, 1H), 3.34 (d, J = 5.2 Hz, 6H), 2.80 - 2.69 (m, 2H), 2.20 - 2.06 (m, 1H), 1.87 - 1.73 (m, 2H), 1.68 - 1.48 (m, 4H), 1.30 - 1.13 (m, 2H). ESI-MS *m/z*: 238.2 $[M+H]^+$.

*4.1.5.5. 5-((Cyclopentylmethyl)amino)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**8k**).* A yellow oil. Yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (s, 1H), 3.89 (s, 1H), 3.32 (d, J = 3.8 Hz, 6H), 2.69 - 2.62 (m, 2H), 1.79 - 1.60 (m, 5H), 1.58 - 1.42 (m, 1H), 1.22 - 1.12 (m, 3H), 0.94 (t, J = 13.3 Hz, 2H). ESI-MS *m/z*: 252.2 $[M+H]^+$.

*4.1.6. General procedure for preparation of compounds (**9a-9z**).*

To a solution of **8a-8z** (2.24 mmol) in DCM (5 mL) was added triethylamine (0.34 g, 3.36 mmol) and stirred at 0 ℃ for 15 min. 3-Chloropropanoyl chloride (0.34 g, 2.69 mmol) was added dropwise and stirred at room temperature for 2-3 h until the reaction was completed. The resulted solution was poured into water (15 mL). The aqueous layer was extracted with DCM (10 mL × 3). The combined organic layer was dried (Na_2SO_4) and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **9a-9z** respectively.

*4.1.6.1. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**9a**).* A white solid. Yield 79%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H), 5.15-5.06 (m, 1H), 4.41 - 4.27 (m, 1H), 3.81 - 3.63 (m, 3H), 3.33 (s, 3H), 3.20 (s, 3H), 2.87 - 2.70 (m, 1H), 2.50 - 2.35 (m, 1H), 1.65 (s, 3H), 1.52 (s, 3H). ESI-MS *m/z*: 314.2 $[M+H]^+$.

*4.1.6.2. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-ethylpropanamide (**9b**).* A yellow oil. Yield 80 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 3.94 - 3.76 (m, 2H), 3.48 (s, 3H), 3.45 - 3.29 (m, 5H), 2.70 (dt, J = 15.5, 7.6 Hz, 1H), 2.53 (dt, J = 16.2, 6.1 Hz, 1H), 1.11 (t, J = 7.2 Hz, 3H). ESI-MS *m/z*: 274.2 $[M+H]^+$.

*4.1.6.3. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-propylpropanamide (**9c**).* A yellow oil. Yield 78 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 1H), 3.93 - 3.81 (m, 1H), 3.79 - 3.63 (m, 3H), 3.46 (s, 3H), 3.38 (s, 3H), 2.76 - 2.62 (m, 1H), 2.60 - 2.46 (m, 1H), 1.56 - 1.44 (m, 2H), 0.89 (t, J = 7.5 Hz, 3H). ESI-MS *m/z*: 288.3 $[M+H]^+$.

*4.1.6.4. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-isopropylpropanamide (**9d**).* A yellow oil. Yield 78 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 1H), 3.93 - 3.81 (m, 2H), 3.79 - 3.63 (m, 1H), 3.46 (s, 3H), 3.35 (s, 3H), 2.72 - 2.58 (m, 1H), 2.55 - 2.43 (m, 1H), 1.15 - 1.10 (m, 6H). ESI-MS *m/z*: 288.3 $[M+H]^+$.

*4.1.6.5. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-butylpropanamide (**9e**).* A yellow oil. Yield 71 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1H), 3.90 - 3.79 (m, 3H), 3.45 (s, 3H), 3.38 (s, 3H), 3.29 - 3.19 (m, 1H), 2.73 - 2.60

(m, 1H), 2.58 - 2.46 (m, 1H), 1.60 - 1.48 (m, 2H), 1.42 - 1.30 (m, 2H), 0.89 (t, J = 7.5 Hz, 3H). ESI-MS *m/z*: 302.4 $[M+H]^+$.

*4.1.6.6. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-isobutylpropanamide (**9f**).* A yellow oil. Yield 73 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 1H), 3.91 - 3.80 (m, 3H), 3.46 (s, 3H), 3.38 (s, 3H), 3.30 - 3.19 (m, 1H), 2.77 - 2.60 (m, 1H), 2.57 - 2.45 (m, 1H), 1.90 - 1.80 (m, 1H), 0.94 (s, 3H), 0.92 (s, 3H). ESI-MS *m/z*: 302.4 $[M+H]^+$.

*4.1.6.7. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-isopentylpropanamide (**9g**).* A yellow oil. Yield 70 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 1H), 3.88 - 3.59 (m, 3H), 3.44 (s, 3H), 3.35 (s, 3H), 3.29 - 3.17 (m, 1H), 2.72 - 2.57 (m, 1H), 2.55 - 2.41 (m, 1H), 1.60 - 1.47 (m, 1H), 1.40 - 1.23 (m, 2H), 0.90 - 0.82 (m, 6H). ESI-MS *m/z*: 316.3 $[M+H]^+$.

*4.1.6.8. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-cyclopropylmethylpropanamide (**9h**).* A yellow oil. Yield 79 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (s, 1H), 3.89 - 3.63 (m, 4H), 3.46 (s, 3H), 3.37 (s, 3H), 2.77 - 2.63 (m, 1H), 2.59 - 2.47 (m, 1H), 0.92 - 0.82 (m, 1H), 0.50 - 0.39 (m, 2H), 0.17 - 0.12 (m, 2H). ESI-MS *m/z*: 300.3 $[M+H]^+$.

*4.1.6.9. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-cyclobutylmethylpropanamide (**9i**).* A yellow oil. Yield 81 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1H), 3.95 - 3.80 (m, 2H), 3.45 (s, 3H), 3.40 - 3.28 (m, 5H), 2.75 - 2.60 (m, 1H), 2.58 - 2.37 (m, 2H), 2.04 - 1.91 (m, 2H), 1.89 - 1.78 (m, 2H), 1.73 - 1.65 (m, 2H). ESI-MS *m/z*: 314.3 $[M+H]^+$.

*4.1.6.10. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-cyclopentylmethylpropanamide (**9j**).* A yellow oil. Yield 80 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 1H), 4.00 - 3.83 (m, 2H), 3.82 - 3.70 (m, 1H), 3.53 (s, 3H), 3.45 (s, 3H), 3.33 - 3.18 (m, 1H), 2.83 - 2.70 (m, 1H), 2.65 - 2.52 (m, 1H), 2.14 - 1.97 (m, 1H), 1.77 - 1.64 (m, 4H), 1.63 - 1.48 (m, 2H), 1.39 - 1.21 (m, 2H). ESI-MS *m/z*: 328.4 $[M+H]^+$.

*4.1.6.11. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-cyclohexylmethylpropanamide (**9k**).* A yellow oil. Yield 76 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 1H), 3.99 - 3.88 (m, 1H), 3.87 - 3.69 (m, 3H), 3.54 (s, 3H), 3.45 (s, 3H), 2.84 - 2.70 (m, 1H), 2.66 - 2.53 (m, 1H), 1.82 - 1.65 (m, 5H), 1.53 - 1.41 (m, 1H), 1.27 - 1.15 (m, 3H), 1.14 - 0.95 (m, 2H). ESI-MS *m/z*: 342.3 $[M+H]^+$.

*4.1.6.12. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-benzylpropanamide (**9l**).* A yellow solid. Yield 83 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.29 (m, 3H), 7.27 - 7.16 (m, 2H), 6.72 (s, 1H), 5.63 (d, J = 14.5 Hz, 1H), 4.02 - 3.86 (m, 1H), 3.84 (d, J = 14.5 Hz, 1H), 3.81 - 3.67 (m, 1H), 3.38 (s, 3H), 3.23 (s, 3H), 2.84 - 2.68(m, 1H), 2.67 - 2.52 (m, 1H). ESI-MS *m/z*: 336.2 $[M+H]^+$.

*4.1.6.13. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(2-methylbenzyl)propanamide (**9m**).* A yellow solid. Yield 81 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 - 7.15 (m, 2H), 7.15 - 7.05 (m, 1H), 7.04 - 6.96 (m, 1H), 6.49 (s, 1H), 5.62 (d, J = 14.5 Hz, 1H), 4.02 - 3.86 (m, 2H), 3.80 - 3.64 (m, 1H), 3.36 (s, 3H), 3.16 (s, 3H), 2.84 - 2.67 (m, 1H), 2.65 - 2.53 (m, 1H), 2.25 (s, 3H). ESI-MS *m/z*: 350.3 $[M+H]^+$.

*4.1.6.14. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3-methyl*benzyl)propanamide (**9n**)*. A yellow solid. Yield 84 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (t, J = 7.5 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 7.01 (s, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.71 (s, 1H), 5.55 (d, J = 14.5 Hz, 1H), 3.94 - 3.81 (m, 1H), 3.78 - 3.64 (m, 2H), 3.33 (s, 3H), 3.20 (s, 3H), 2.78 - 2.65 (m, 1H), 2.62 - 2.50 (m, 1H), 2.28 (s, 3H). ESI-MS *m/z*: 350.3 $[M+H]^+$.

*4.1.6.15. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*4-methyl*benzyl)propanamide (**9o**)*. A yellow solid. Yield 81 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 -7.19 (m, 2H), 7.14 -7.08 (m, 2H), 6.51 (s, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.71 (s, 1H), 5.57 (d, J = 14.6 Hz, 1H), 3.91 - 3.79 (m, 1H), 3.73 - 3.60 (m, 2H), 3.34 (s, 3H), 3.20 (s, 3H), 2.80 - 2.68 (m, 1H), 2.65 - 2.53 (m, 1H), 2.27 (s, 3H). ESI-MS *m/z*: 350.3 $[M+H]^+$.

*4.1.6.16. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3,5-dimethyl*benzyl)propanamide (**9p**)*. A yellow solid. Yield 81 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.83 - 6.77 (m, 2H), 6.70 (s, 1H), 5.57 (d, J = 14.4 Hz, 1H), 3.97 - 3.82 (m, 1H), 3.77 - 3.63 (m, 2H), 3.36 (s, 3H), 3.22 (s, 3H), 2.80 - 2.66 (m, 1H), 2.64 - 2.52 (m, 1H), 2.26 (s, 6H). ESI-MS *m/z*: 364.2 $[M+H]^+$.

*4.1.6.17. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*2-fluoro*benzyl)propanamide (**9q**)*. A yellow solid. Yield 80 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 - 7.36 (m, 1H), 7.35 - 7.21 (m, 1H), 7.16 - 7.08 (m, 1H), 7.05 - 6.94 (m, 2H), 5.31 (d, J = 14.5 Hz, 1H), 4.28 (d, J = 14.5 Hz, 1H), 3.95 - 3.80 (m, 1H), 3.79 - 3.64 (m, 1H), 3.36 (s, 3H), 3.31 (s, 3H), 2.84 - 2.69 (m, 1H), 2.66 - 2.51 (m, 1H). ESI-MS *m/z*: 354.2 $[M+H]^+$.

*4.1.6.18. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3-fluoro*benzyl)propanamide (**9r**).* A yellow solid. Yield 78 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.22 (m, 1H), 7.06 - 6.93 (m, 3H), 6.88 (s, 1H), 5.56 (d, J = 14.7 Hz, 1H), 4.02 - 3.83 (m, 2H), 3.81 - 3.66 (m, 1H), 3.38 (s, 3H), 3.29 (s, 3H), 2.86 - 2.69 (m, 1H), 2.69 - 2.52 (m, 1H). ESI-MS *m/z*: 354.2 $[M+H]^+$.

*4.1.6.19. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*4-fluoro*benzyl)propanamide (**9s**).* A yellow solid. Yield 74 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 - 7.17 (m, 2H), 7.07 - 6.94 (m, 2H), 6.77 (s, 1H), 5.53 (d, J = 14.7 Hz, 1H), 4.02 - 3.82 (m, 2H), 3.80 - 3.66 (m, 1H), 3.38 (s, 3H), 3.28 (s, 3H), 2.84 - 2.68 (m, 1H), 2.66 - 2.51 (m, 1H). ESI-MS *m/z*: 354.2 $[M+H]^+$.

*4.1.6.20. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*2-chloro*benzyl)propanamide (**9t**).* A yellow solid. Yield 75 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.36 (m, 1H), 7.33 - 7.29 (m, 1H), 7.24 - 7.17 (m, 2H), 6.92 (s, 1H), 5.39 (d, J = 14.7 Hz, 1H), 4.35 (d, J = 14.7 Hz, 1H), 3.95 - 3.80 (m, 1H), 3.78 - 3.63 (m, 1H), 3.33 (s, 3H), 3.25 (s, 3H), 2.84 - 2.67 (m, 1H), 2.67 - 2.51 (m, 1H). ESI-MS *m/z*: 370.1 $[M+H]^+$.

*4.1.6.21. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3-chloro*benzyl)propanamide (**9u**).* A yellow solid. Yield 77 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 - 7.22 (m, 3H), 7.18 - 7.10 (m, 1H), 6.94 (s, 1H), 5.50 (d, J = 14.8 Hz, 1H), 3.96 - 3.86 (m, 2H), 3.80 - 3.66 (m, 1H), 3.37 (s, 3H), 3.30 (s, 3H), 2.87 - 2.69 (m, 1H), 2.68 - 2.52 (m, 1H). ESI-MS *m/z*: 370.1 $[M+H]^+$.

*4.1.6.22. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*4-chloro*benzyl)propanamide (**9v**).* A yellow solid. Yield 76 %. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.32 - 7.26 (m, 2H), 7.23 - 7.15 (m, 2H), 6.85 (s, 1H), 5.50 (d, J = 14.7 Hz, 1H), 3.99 - 3.85 (m, 2H), 3.79 - 3.68 (m, 1H), 3.38 (s, 3H), 3.29 (s, 3H), 2.84 - 2.70 (m, 1H), 2.66 - 2.54 (m, 1H). ESI-MS *m/z*: 370.1 $[M+H]^+$.

*4.1.6.23. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*2-(trifluoromethyl)*benzyl)propanamide (**9w**).* A yellow solid. Yield 80 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 - 7.44 (m, 3H), 7.36 - 7.30 (m, 1H), 6.90 (s, 1H), 5.41 (d, J = 15.7 Hz, 1H), 4.43 (d, J = 15.6 Hz, 1H), 3.96 - 3.81 (m, 1H), 3.74 - 3.64 (m, 1H), 3.29 (s, 3H), 3.21 (s, 3H), 2.85 - 2.68 (m, 1H), 2.68 - 2.52 (m, 1H). ESI-MS *m/z*: 404.2 $[M+H]^+$.

*4.1.6.24. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3-(trifluoromethyl)*benzyl)propanamide (**9x**).* A yellow solid. Yield 79 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 - 7.51 (m, 1H), 7.50 - 7.43 (m, 3H), 6.83 (s, 1H), 5.54 (d, J = 14.8 Hz, 1H), 4.07 - 3.85 (m, 2H), 3.79 - 3.64 (m, 1H), 3.36 (s, 3H), 3.27 (s, 3H), 2.87 - 2.67 (m, 1H), 2.66 - 2.51 (m, 1H). ESI-MS *m/z*: 404.2 $[M+H]^+$.

*4.1.6.25. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*2-methoxy*benzyl)propanamide (**9y**).* A yellow solid. Yield 75 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 - 7.14 (m, 2H), 6.90 - 6.83 (m, 1H), 6.79 - 6.73 (m, 2H), 5.21 (d, J = 14.3 Hz, 1H), 4.20 (d, J = 14.3 Hz, 1H), 3.89 - 3.80 (m, 1H), 3.70 - 3.62 (m, 4H), 3.30 (s, 3H), 3.18 (s, 3H), 2.75 - 2.62 (m, 1H), 2.60 - 2.46 (m, 1H). ESI-MS *m/z*: 365.3 $[M+H]^+$.

*4.1.6.26. 3-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(*3-methoxy*benzyl)propanamide (**9z**).* A yellow solid. Yield 79 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 - 7.18 (m, 1H), 6.86 - 6.73 (m, 4H), 5.63 (d, J = 14.5 Hz, 1H), 4.03 -

3.88 (m, 1H), 3.84 - 3.69 (m, 5H), 3.39 (s, 3H), 3.25 (s, 3H), 2.83 - 2.68 (m, 1H), 2.67 - 2.54 (m, 1H). ESI-MS *m/z*: 365.3 $[M+H]^+$.

*4.1.6.27. 2-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide(**9aa**).*

To a solution of **8a** (0.5 g, 2.24 mmol) in DCM (5 mL) was added triethylamine (0.34 g, 3.36 mmol) and stirred at 0 ℃ for 15 min. 2-chloroacetyl chloride (0.31 g, 2.69 mmol) was added dropwise and stirred at room temperature for 2 h. The resulted solution was poured into water (15 mL) .The aqueous layer was extracted with DCM (10 mL × 2). The combined organic layer was dried (Na_2SO_4) and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **9aa** as a yellow oil. Yield 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 5.14 (t, J = 7.5 Hz, 1H), 4.62 - 4.49 (m, 1H), 4.01 - 3.85 (m, 2H), 3.83 - 3.70 (m, 1H), 3.47 (s, 3H), 3.39 (s, 3H), 1.72 (s, 3H), 1.58 (s, 3H). ESI-MS *m/z*: 300.2 $[M+H]^+$.

*4.1.6.28. 4-Chloro-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide(**9ab**).*

To a solution of **8a** (0.5 g, 2.24 mmol) in DCM (5 mL) was added butanamide (0.38 g, 3.36 mmol) and stirred at 0 ℃ for 15 min. 4- chlorobutanoyl chloride (0.31 g, 2.69 mmol) was added dropwise and stirred at room temperature for 2 h. The resulted solution was poured into water (15 mL) .The aqueous layer was extracted with DCM (10 mL × 2). The combined organic layer was dried (Na_2SO_4) and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **9ab** as a yellow oil. Yield 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1H), 5.19 - 5.07 (m, 1H), 4.70 - 4.56 (m, 1H), 3.68 - 3.53 (m, 3H), 3.45 (s, 3H), 3.40 (s, 3H), 2.65 - 2.49

(m, 1H), 2.51 - 2.34 (m, 1H), 2.17 - 2.02 (m, 2H), 1.71 (s, 3H), 1.57 (s, 3H). ESI-MS *m/z*: 328.3 $[M+H]^+$.

*4.1.7. General procedure for preparation of compounds (**11-59**).*

To a solution of **9** (0.64 mmol) in CH_3CN (5 mL) was added piperazine (0.16 g, 1.91 mmol), potassium iodide (0.2 g, 1.27mmol) and potassium carbonate (0.18 g, 1.27 mmol). The mixture was stirred at room temperature for 10-20 h until the reaction was completed. The resulted solution was poured into water (15 mL) and extracted with ethyl acetate (15 mL × 2). The organic layer was dried (Na_2SO_4) and filtered. The solvent was evaporated under vacuum to give crude product **10**. To a solution of **10** (0.28 mmol) in DCM (4 mL) was added triethylamine (0.06 g, 0.55 mmol), different acid (0.33 mmol) and EDCI (0.11 g, 0.55 mmol). The mixture was stirred at room temperature for 15-20 h until the reaction was completed. The reaction was poured into water (5 mL) and the mixture was extracted with DCM (20 mL×3). The combined organic layer was dried over anhydrous sodium sulfate and filtered. After removal of solvent, the crude product was purified by silica gel chromatography to give **11-59** respectively.

*4.1.7.1. 3-(4-Benzoylpiperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**11**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.56 - 7.41 (m, 5H), 5.30 - 5.17 (m, 1H), 4.58 - 4.45 (m, 1H), 3.94 - 3.68 (m, 3H), 3.52 - 3.43 (m, 5H), 3.38 (s, 3H), 2.84 - 2.70 (m, 2H), 2.63 - 2.34 (m, 6H), 1.76 (s, 3H), 1.64 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.48, 172.25, 162.96, 152.62, 145.88, 138.16, 136.67, 131.11, 129.71, 128.02, 120.21, 116.13, 54.80, 54.12, 53.56, 46.41, 37.48, 32.02, 28.79, 25.87,

17.92. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{34}N_5O_4$ $[M+H]^+$ 468.2611, found 468.2606.

*4.1.7.2. 3-(4-(4-Methylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**12**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.24 - 7.12 (m, 4H), 5.04 - 4.95 (m, 1H), 4.51 - 4.38 (m, 1H), 3.78 - 3.40 (m, 5H), 3.40 - 3.11 (m, 10H), 3.08 - 2.63 (m, 3H), 2.55 - 2.39 (m, 1H), 2.31 (s, 3H), 1.61 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.48, 169.81, 160.95, 150.66, 144.04, 141.04, 137.75, 130.49, 129.25, 127.11, 118.26, 113.72, 52.72, 52.18, 51.80, 45.36, 37.10, 28.31, 28.20, 25.46, 21.22, 17.63. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{36}N_5O_4$ $[M+H]^+$ 482.2767, found 482.2765.

*4.1.7.3. 3-(4-(4-Ethylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**13**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.46 - 7.32 (m, 4H), 5.32 - 5.20 (m, 1H), 4.61 - 4.47 (m, 1H), 3.97 - 3.66 (m, 3H), 3.61 - 3.46 (m, 5H), 3.41 (s, 3H), 2.84 - 2.69 (m, 4H), 2.66 - 2.37 (m, 6H), 1.78 (s, 3H), 1.66 (s, 3H), 1.32 (t, J = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.86, 170.00, 161.21, 150.89, 147.63, 144.20, 138.05, 130.82, 128.36, 127.51, 118.50, 114.03, 53.20, 52.60, 52.52, 45.76, 37.35, 28.81, 28.62, 28.58, 25.68, 17.86, 15.31. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{38}N_5O_4$ $[M+H]^+$ 496.2924, found 496.2921.

*4.1.7.4. 3-(4-(4-Isopropylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**14**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.36 - 7.24 (m, 4H), 5.13 - 5.04 (m, 1H), 4.59 - 4.49 (m, 1H), 3.80 - 3.47 (m, 5H), 3.47 - 3.15 (m, 9H), 3.13 - 2.66

(m, 5H), 2.64 - 2.47 (m, 1H), 1.70 (s, 3H), 1.54 (s, 3H), 1.26 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.79, 169.98, 161.18, 152.16, 150.86, 144.17, 138.01, 130.95, 127.45, 126.93, 118.46, 113.99, 53.10, 52.51, 52.43, 45.70, 37.30, 34.11, 28.55, 28.54, 25.64, 23.74, 17.83. HRMS (ESI-TOF) *m/*z calc'd for $C_{28}H_{40}N_5O_4$ $[M+H]^+$ 510.3080, found 510.3078.

*4.1.7.5. 3-(4-(4-Methoxybenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**15**)*. A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.34 - 7.29 (m, 2H), 7.26 - 7.19 (m, 2H), 5.13 - 5.02 (m, 1H), 4.60 - 4.47 (m, 1H), 3.78 - 3.62 (m, 2H), 3.62 - 3.48 (m, 3H), 3.48 - 3.33 (m, 7H), 3.32 - 3.17 (m, 2H), 3.13 - 2.94 (m, 2H), 2.91 - 2.64 (m, 2H), 2.62 - 2.49 (m, 1H), 2.39 (s, 3H), 1.70 (s, 3H), 1.54 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.62, 169.96, 161.75, 161.18, 150.92, 144.22, 137.99, 129.56, 125.59, 118.57, 114.16, 113.77, 55.49, 53.13, 52.50, 52.47, 45.69, 37.33, 28.59, 28.58, 25.70, 17.88. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{36}N_5O_5$ $[M+H]^+$ 498.2716, found 498.2721.

*4.1.7.6. 3-(4-(4-Fluorobenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**16**)*. A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.64 - 7.50 (m, 2H), 7.31 - 7.17 (m, 2H), 5.25 - 5.12 (m, 1H), 4.51 - 4.36 (m, 1H), 4.12 - 3.68 (m, 4H), 3.59 - 3.37 (m, 9H), 3.36 - 3.29 (m, 4H), 2.99 - 2.81 (m, 1H), 2.74 - 2.57 (m, 1H), 1.71 (s, 3H), 1.59 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.09, 169.61, 163.89, 161.14, 150.72, 144.05, 138.06, 129.62, 129.50, 118.18, 115.93, 113.84, 52.98, 52.26, 51.88, 45.65, 37.20, 28.42, 28.31, 25.49, 17.67. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{33}FN_5O_4$ $[M+H]^+$ 486.2517, found 486.2515.

*4.1.7.7. 3-(4-(4-Chlorobenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**17**).* A white solid. Yield 43 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.53 - 7.43 (m, 4H), 5.23 - 5.10 (m, 1H), 4.48 - 4.34 (m, 1H), 4.03 - 3.61 (m, 4H), 3.59 - 3.39 (m, 8H), 3.38 - 3.31 (m, 5H), 2.93 - 2.76 (m, 1H), 2.71 - 2.54 (m, 1H), 1.69 (s, 3H), 1.57 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.96, 169.38, 161.12, 150.80, 144.10, 137.98, 136.97, 131.99, 129.11, 128.75, 118.37, 113.92, 52.90, 52.14, 52.12, 45.60, 37.24, 28.48, 28.45, 25.60, 17.78. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{33}ClN_5O_4$ $[M+H]^+$ 502.2221, found 502.2216.

*4.1.7.8. 3-(4-(4-Bromobenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**18**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.73 - 7.63 (m, 2H), 7.49 - 7.39 (m, 2H), 5.25 - 5.11 (m, 1H), 4.51 - 4.36 (m, 1H), 4.05 - 3.64 (m, 4H), 3.61 - 3.40 (m, 8H), 3.39 - 3.31 (m, 5H), 2.97 - 2.80 (m, 1H), 2.74 - 2.57 (m, 1H), 1.72 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.94, 169.34, 161.08, 150.76, 144.07, 137.89, 132.48, 132.00, 128.82, 125.12, 118.33, 113.87, 52.79, 51.97, 51.96, 45.51, 37.17, 28.41, 28.36, 25.53, 17.71. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{33}BrN_5O_4$ $[M+H]^+$ 546.1716, found 546.1712.

*4.1.7.9. 3-(4-(4-(Trifluoromethyl)benzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**19**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 - 7.62 (m, 2H), 7.55 - 7.45 (m, 2H), 7.34 (s, 1H), 5.07 - 4.97 (m, 1H), 4.54 - 4.41 (m, 1H), 3.78 - 3.44 (m, 6H), 3.41 - 3.25 (m, 9H), 3.08 - 2.74 (m, 3H), 2.61 - 2.44 (m, 1H), 1.65 (s, 3H), 1.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.00, 168.96, 161.20, 150.85, 144.09, 138.08, 137.31,

132.73, 127.66, 125.99, 123.50, 118.42, 114.03, 53.00, 52.23, 52.21, 45.73, 37.31, 28.58, 28.56, 25.64, 17.83. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{33}F_3N_5O_4$ $[M+H]^+$ 536.2485, found 536.2481.

*4.1.7.10. 3-(4-(4-Nitrobenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**20**)*. A white solid. Yield 44 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 - 8.28 (m, 2H), 7.85 (s, 1H), 7.79 - 7.68 (m, 2H), 5.22 - 5.11 (m, 1H), 4.48 - 4.34 (m, 1H), 3.96 - 3.60 (m, 4H), 3.61 - 3.38 (m, 8H), 3.36 - 3.27 (m, 5H), 2.94 - 2.77 (m, 1H), 2.72 - 2.55 (m, 1H), 1.70 (s, 3H), 1.58 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.03, 168.07, 161.18, 150.79, 148.89, 144.02, 139.87, 138.04, 128.31, 124.09, 118.33, 113.99, 52.89, 52.01, 51.97, 45.67, 37.24, 28.48, 28.47, 25.57, 17.76. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{33}N_6O_6$ $[M+H]^+$ 513.2462, found 513.2456.

*4.1.7.11. 3-(4-(4-Cyanobenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**21**)*. A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 - 7.83 (m, 3H), 7.73 - 7.64 (m, 2H), 5.25 - 5.13 (m, 1H), 4.50 - 4.36 (m, 1H), 4.01 - 3.62 (m, 4H), 3.60 - 3.39 (m, 8H), 3.37 - 3.32 (m, 5H), 2.95 - 2.79 (m, 1H), 2.74 - 2.57 (m, 1H), 1.72 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.02, 168.34, 161.17, 150.76, 144.02, 138.05, 132.67, 127.89, 118.27, 117.70, 114.40, 113.94, 52.91, 52.00, 51.98, 45.68, 37.24, 28.47, 28.45, 25.56, 17.75. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{33}N_6O_4$ $[M+H]^+$ 493.2563, found 493.2562.

*4.1.7.12. 3-(4-(Furan-2-carbonyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**22**)*. A white solid. Yield 41 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 - 7.44 (m, 1H), 7.37 (s, 1H), 7.12 -

7.05 (m, 1H), 6.53 - 6.45 (m, 1H), 5.12 - 5.00 (m, 1H), 4.79 - 4.57 (m, 1H), 4.58 - 4.43 (m, 1H), 3.78 - 3.60 (m, 2H), 3.60 - 3.46 (m, 3H), 3.46 - 3.13 (m, 9H), 3.13 - 2.69 (m, 3H), 2.63 - 2.46 (m, 1H), 1.67 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.96, 161.13, 158.68, 150.83, 146.70, 144.51, 144.17, 137.96, 118.40, 118.39, 113.91, 111.84, 53.08, 52.52, 52.47, 45.60, 37.26, 28.48, 28.42, 25.60, 17.78. HRMS (ESI-TOF) *m/*z calc'd for $C_{23}H_{32}N_5O_5$ $[M+H]^+$ 458.2403, found 458.2397.

*4.1.7.13. 3-(4-Nicotinoylpiperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**23**).* A white solid. Yield 43 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 - 7.44 (m, 1H), 7.37 (s, 1H), 7.12 - 7.05 (m, 1H), 6.53 - 6.45 (m, 1H), 5.12 - 5.00 (m, 1H), 4.79 - 4.57 (m, 1H), 4.58 - 4.43 (m, 1H), 3.78 - 3.60 (m, 2H), 3.60 - 3.46 (m, 3H), 3.46 - 3.13 (m, 9H), 3.13 - 2.69 (m, 3H), 2.63 - 2.46 (m, 1H), 1.67 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.96, 161.13, 158.68, 150.83, 146.70, 144.51, 144.17, 137.96, 118.40, 118.39, 113.91, 111.84, 53.08, 52.52, 52.47, 45.60, 37.26, 28.48, 28.42, 25.60, 17.78. HRMS (ESI-TOF) *m/*z calc'd for $C_{24}H_{33}N_6O_4$ $[M+H]^+$ 469.2563, found 469.2559.

*4.1.7.14. 3-(4-Isonicotinoylpiperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**24**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 - 8.84 (m, 2H), 7.92 - 7.84 (m, 3H), 5.25 - 5.14 (m, 1H), 4.51 - 4.37 (m, 1H), 3.98 - 3.63 (m, 5H), 3.61 - 3.40 (m, 9H), 3.37 - 3.34 (m, 3H), 2.97 - 2.80 (m, 1H), 2.73 - 2.62 (m, 1H), 1.73 (s, 3H), 1.61 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 172.19, 167.96, 163.33, 152.57, 148.47, 147.91, 146.54, 138.73, 124.80, 119.78, 115.35, 54.26, 52.58, 52.54, 46.89, 37.62, 29.20, 28.86, 25.81, 17.87. HRMS (ESI-TOF) *m/*z calc'd for $C_{24}H_{33}N_6O_4$ $[M+H]^+$ 469.2563, found 469.2558.

*4.1.7.15. 3-(Benzo[d][1,3]dioxole-5-carbonyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**25**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 - 8.84 (m, 2H), 7.92 - 7.84 (m, 3H), 5.25 - 5.14 (m, 1H), 4.51 - 4.37 (m, 1H), 3.98 - 3.63 (m, 5H), 3.61 - 3.40 (m, 9H), 3.37 - 3.34 (m, 3H), 2.97 - 2.80 (m, 1H), 2.73 - 2.62 (m, 1H), 1.73 (s, 3H), 1.61 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 172.19, 167.96, 163.33, 152.57, 148.47, 147.91, 146.54, 138.73, 124.80, 119.78, 115.35, 54.26, 52.58, 52.54, 46.89, 37.62, 29.20, 28.86, 25.81, 17.87. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{34}N_5O_6$ $[M+H]^+$ 512.2509, found 512.2504.

*4.1.7.16. 3-(4-(Cyclopropanecarbonyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)propanamide (**26**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 5.29 - 5.16 (m, 1H), 4.57 - 4.43 (m, 1H), 3.93 - 3.74 (m, 3H), 3.67 - 3.52 (m, 2H), 3.48 (s, 3H), 3.38 (s, 3H), 2.80 - 2.66 (m, 2H), 2.61 - 2.31 (m, 6H), 2.06 - 1.91 (m, 1H), 1.76 (s, 3H), 1.63 (s, 3H), 0.95 - 0.80 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ 174.50, 174.28, 163.00, 152.66, 145.95, 138.23, 120.18, 116.11, 54.87, 54.37, 53.64, 46.40, 46.26, 42.97, 37.52, 32.00, 28.80, 25.87, 17.91, 11.58, 7.98. HRMS (ESI-TOF) *m/*z calc'd for $C_{22}H_{34}N_5O_4$ $[M+H]^+$ 432.2611, found 432.2607.

*4.1.7.17. 2-(4-(4-Methylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**27**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.34 - 7.21 (m, 4H), 5.22 - 5.05 (m, 1H), 4.48 - 4.33 (m, 1H), 4.29 - 4.17 (m, 1H), 3.94 - 3.75 (m, 5H), 3.47 - 3.33 (m, 6H), 3.33 - 3.21 (m, 5H), 2.34 (s, 3H), 1.66 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 172.67, 166.14, 162.79, 152.48, 147.18, 142.46, 139.43, 132.28,

130.44, 128.41, 119.07, 113.32, 57.67, 53.50, 46.96, 37.72, 28.87, 25.82, 21.45, 17.92. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{34}N_5O_4$ $[M+H]^+$ 468.2611, found 468.2608.

*4.1.7.18. 2-(4-(4-Ethylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**28**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.37 - 7.21 (m, 4H), 5.17 - 5.05 (m, 1H), 4.47 - 4.32 (m, 1H), 4.27 - 4.15 (m, 1H), 3.94 - 3.72 (m, 5H), 3.42 - 3.18 (m, 11H), 2.63 (q, J = 7.6 Hz, 2H), 1.65 (s, 3H), 1.53 (s, 3H), 1.18 (t, J = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 172.71, 166.15, 162.82, 152.50, 148.80, 147.19, 139.45, 132.54, 129.34, 128.54, 119.07, 113.35, 57.70, 53.53, 47.00, 37.72, 29.71, 28.87, 25.82, 17.92, 15.90. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{36}N_5O_4$ $[M+H]^+$ 482.2767, found 482.2765.

*4.1.7.19. 2-(4-(4-Isopropylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**29**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.40 - 7.31 (m, 4H), 5.22 - 5.10 (m, 1H), 4.49 - 4.35 (m, 1H), 4.29 - 4.17 (m, 1H), 3.96 - 3.77 (m, 5H), 3.46 - 3.26 (m, 11H), 3.04 - 2.86 (m, 1H), 1.69 (s, 3H), 1.58 (s, 3H), 1.24 (d, J = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, MeOD) δ 172.72, 166.18, 162.85, 153.38, 152.52, 147.18, 139.49, 132.70, 128.59, 127.95, 119.08, 113.40, 57.73, 53.57, 47.04, 37.71, 35.36, 28.87, 25.82, 24.17, 17.92. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{38}N_5O_4$ $[M+H]^+$ 496.2924, found 496.2922.

*4.1.7.20. 2-(4-(4-Methoxybenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**30**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.49 - 7.39 (m, 2H), 7.06 - 6.96 (m, 2H), 5.24 - 5.12 (m, 1H), 4.51 - 4.37 (m, 1H), 4.30 - 4.18 (m, 1H), 4.01 - 3.77

(m, 8H), 3.45 - 3.30 (m, 11H), 1.71 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 172.76, 166.19, 163.00, 162.97, 152.59, 147.32, 139.85, 130.48, 126.73, 118.66, 115.12, 113.21, 57.71, 56.06, 53.51, 47.03, 37.93, 29.04, 25.77, 17.89. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{34}N_5O_5$ $[M+H]^+$ 484.2560, found 484.2556.

*4.1.7.21. 4-(4-(4-Methylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**31**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.38 - 7.25 (m, 4H), 5.26 - 5.14 (m, 1H), 4.55 - 4.41 (m, 1H), 3.89 - 3.48 (m, 5H), 3.44 (s, 3H), 3.35 (s, 3H), 2.69 - 2.43 (m, 6H), 2.40 (s, 3H), 2.38 - 2.27 (m, 1H), 2.26 - 2.11 (m, 1H), 1.87 - 1.75 (m, 2H), 1.73 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 175.70, 172.48, 162.96, 152.61, 145.86, 141.67, 138.10, 133.50, 130.24, 128.19, 120.26, 116.23, 58.36, 53.91, 53.85, 46.39, 37.47, 32.10, 28.76, 25.84, 22.78, 21.41, 17.88. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{38}N_5O_4$ $[M+H]^+$ 496.2924, found 496.2921.

*4.1.7.22. 4-(4-(4-Ethylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**32**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.43 - 7.30 (m, 4H), 5.27 - 5.18 (m, 1H), 4.58 - 4.45 (m, 1H), 3.91 - 3.66 (m, 3H), 3.61 - 3.43 (m, 5H), 3.37 (s, 3H), 2.73 (q, J = 7.7 Hz, 2H), 2.61 - 2.42 (m, 6H), 2.41 - 2.14 (m, 2H), 1.88 - 1.77 (m, 2H), 1.75 (s, 3H), 1.62 (s, 3H), 1.29 (t, J = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 175.68, 172.39, 162.91, 152.59, 147.91, 145.83, 138.03, 133.84, 129.11, 128.31, 120.33, 116.25, 58.41, 54.05, 53.95, 46.36, 37.49, 32.14, 29.68, 28.79, 25.89, 22.93, 17.93, 16.01. HRMS (ESI-TOF) *m/*z calc'd for $C_{28}H_{40}N_5O_4$ $[M+H]^+$ 510.3080, found 510.3079.

*4.1.7.23. 4-(4-(4-Isopropylbenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**33**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.37 - 7.30 (m, 4H), 5.23 - 5.13 (m, 1H), 4.52 - 4.42 (m, 1H), 3.84 - 3.68 (m, 3H), 3.53 - 3.40 (m, 5H), 3.33 (s, 3H), 3.01 - 2.89 (m, 1H), 2.53 - 2.32 (m, 6H), 2.36 - 2.25 (m, 2H), 2.22 - 2.10 (m, 1H), 1.81 - 1.73 (m, 1H), 1.71 (s, 3H), 1.58 (s, 3H), 1.27 (d, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, MeOD) δ 175.80, 172.46, 162.94, 152.63, 152.48, 145.83, 138.05, 134.06, 128.32, 127.70, 120.33, 116.30, 58.47, 54.25, 53.82, 46.37, 37.47, 35.31, 32.19, 28.78, 25.88, 24.28, 23.09, 17.92. HRMS (ESI-TOF) *m/*z calc'd for $C_{29}H_{42}N_5O_4$ $[M+H]^+$ 524.3237, found 524.3232.

*4.1.7.24. 4-(4-(4-Methoxybenzoyl)piperazin-1-yl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-N-(3-methylbut-2-en-1-yl)acetamide (**34**).* A white solid. Yield 46 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.47 - 7.40 (m, 2H), 7.08 - 6.99 (m, 2H), 5.29 - 5.17 (m, 1H), 4.59 - 4.45 (m, 1H), 3.92 - 3.79 (m, 4H), 3.78 - 3.51 (m, 4H), 3.47 (s, 3H), 3.37 (s, 3H), 2.58 - 2.39 (m, 6H), 2.38 - 2.29 (m, 1H), 2.28 - 2.16 (m, 1H), 1.86 - 1.78 (m, 2H), 1.75 (s, 3H), 1.63 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 175.70, 172.22, 162.91, 162.55, 152.59, 145.81, 138.03, 130.21, 128.37, 120.33, 116.27, 114.89, 58.44, 55.94, 53.97, 53.94, 46.36, 37.49, 32.15, 28.79, 25.88, 22.98, 17.93. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{38}N_5O_5$ $[M+H]^+$ 512.2873, found 512.2873.

*4.1.7.25. N-ethyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**35**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.34 (s, 4H), 3.88 - 3.57 (m, 3H), 3.49 - 3.37 (m, 4H), 3.37 - 3.23 (m, 5H), 3.05 - 2.85 (m, 1H), 2.83 - 2.64 (m, 2H), 2.62 - 2.23 (m,

6H), 1.27 (d, *J* = 7.5 Hz, 6H), 1.10 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.24, 171.16, 161.79, 151.39, 151.15, 144.46, 132.67, 126.94, 126.33, 115.04, 53.46, 52.81, 52.46, 43.12, 36.15, 33.94, 30.68, 27.43, 22.86, 11.80. HRMS (ESI-TOF) *m/*z calc'd for $C_{25}H_{36}N_5O_4$ $[M+H]^+$ 470.2767, found 470.2768.

*4.1.7.26. N-propyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**36**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.38 - 7.32 (m, 4H), 3.80 - 3.64 (m, 3H), 3. 50 - 3.41 (m, 5H), 3.34 (s, 3H), 3.28 - 3.16 (m, 1H), 3.02 - 2.91 (m, 1H), 2.74 - 2.67 (m, 2H), 2.57 - 2.29 (m, 6H), 1.60 - 1.46 (m, 2H), 1.28 (s, 3H), 1.26 (s, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.72, 172.29, 162.93, 152.64, 152.36, 145.68, 134.00, 128.28, 127.63, 116.60, 54.81, 54.18, 53.72, 51.13, 37.51, 35.21, 31.99, 28.80, 24.25, 21.86, 11.58. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{38}N_5O_4$ $[M+H]^+$ 484.2924, found 484.2923.

*4.1.7.27. N-isopropyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**37**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1H), 7.25 - 7.21 (m, 2H), 7.19 - 7.13 (m, 2H), 4.86 - 4.69 (m, 1H), 3.80 - 3.47 (m, 2H), 3.46 - 3.24 (m, 8H), 2.91 - 2.79 (m, 1H), 2.67 - 2.60 (m, 2H), 2.45 - 2.12 (m, 6H), 1.19 (s, 3H), 1.17 (s, 3H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.05, 169.56, 161.00, 150.18, 149.85, 142.56, 132.14, 126.27, 125.64, 111.93, 52.96, 52.51, 52.01, 46.08, 36.52, 33.12, 31.02, 27.80, 22.97, 20.66, 18.65. HRMS (ESI-TOF) *m/*z calc'd for $C_{26}H_{38}N_5O_4$ $[M+H]^+$ 484.2924, found 484.2923.

*4.1.7.28. N-butyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**38**).* A white solid. Yield 46 %. ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.33 - 7.29 (m, 3H), 7.29 - 7.17 (m, 2H), 3.86 - 3.57 (m, 3H), 3.51 - 3.34 (m, 8H), 3.29 - 3.17 (m, 1H), 2.99 - 2.84 (m, 1H), 2.79 - 2.67 (m, 2H), 2.53 - 2.22 (m, 6H), 1.50 - 1.35 (m, 2H), 1.34 - 1.25 (m, 5H), 1.24 (s, 3H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.08, 170.44, 160.92, 150.95, 150.71, 142.48, 133.00, 127.14, 126.49, 116.37, 53.80, 52.89, 48.21, 47.59, 37.36, 34.00, 31.35, 29.96, 28.56, 23.82, 19.95, 13.83. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{40}N_5O_4$ $[M+H]^+$ 490.3080, found 490.3077.

*4.1.7.29. N-isobutyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**39**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 - 7.27 (m, 2H), 7.25 - 7.17 (m, 3H), 3.86 - 3.54 (m, 3H), 3.46 - 3.28 (m, 8H), 3.09 - 2.98 (m, 1H), 2.97 - 2.82 (m, 1H), 2.79 - 2.64 (m, 2H), 2.59 - 2.22 (m, 6H), 1.76 - 1.57 (m, 1H), 1.24 (s, 3H), 1.21 (s, 3H), 0.93 - 0.84 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.46, 170.51, 160.85, 150.94, 150.79, 142.34, 132.99, 127.21, 126.52, 116.77, 55.23, 53.87, 53.45, 47.70, 37.45, 34.05, 31.37, 28.61, 27.25, 23.84, 20.10, 20.01. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{40}N_5O_4$ $[M+H]^+$ 490.3080, found 490.3075.

*4.1.7.30. N-isopentyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**40**).* A white solid. Yield 45 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 - 7.28 (m, 3H), 7.27 - 7.19 (m, 2H), 3.89 - 3.56 (m, 3H), 3.55 - 3.34 (m, 8H), 3.32 - 3.14 (m, 1H), 2.99 - 2.86 (m, 1H), 2.80 - 2.67 (m, 2H), 2.65 - 2.57 (m, 1H), 2.50 - 2.42 (m, 2H), 2.42 - 2.20 (m, 3H), 1.64 - 1.48 (m, 1H), 1.45 - 1.30 (m, 2H), 1.25 (d, J = 6.9 Hz, 6H), 0.94 - 0.85 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.03, 170.47, 160.93, 150.95, 150.74, 142.52, 132.95, 127.14, 126.49, 116.33, 53.76, 52.77, 47.05, 41.99, 37.38, 36.59, 33.99, 31.30, 28.58, 26.03, 23.81,

22.60, 22.47. HRMS (ESI-TOF) *m/*z calc'd for $C_{28}H_{42}N_5O_4$ $[M+H]^+$ 512.3237, found 512.3234.

*4.1.7.31. N-(cyclopropylmethyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**41**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.33 - 7.21 (m, 4H), 3.83 - 3.60 (m, 3H), 3.49 - 3.38 (m, 5H), 3.38 (s, 3H), 3.09 - 2.97 (m, 1H), 2.95 - 2.87 (m, 1H), 2.76 - 2.68 (m, 2H), 2.52 - 2.24 (m, 6H), 1.26 (s, 3H), 1.24 (s, 3H), 0.95 - 0.79 (m, 1H), 0.53 - 0.37 (m, 2H), 0.17 - 0.12 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.07, 170.31, 160.80, 150.88, 150.60, 142.86, 132.78, 126.99, 126.37, 115.85, 53.63, 53.39, 52.64, 51.82, 47.46, 41.79, 37.22, 33.85, 31.13, 28.40, 23.69, 9.54, 4.11, 2.84. HRMS (ESI-TOF) *m/*z calc'd for $C_{27}H_{38}N_5O_4$ $[M+H]^+$ 496.2924, found 496.2921.

*4.1.7.32. N-(cyclobutylmethyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**42**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.31 - 7.22 (m, 4H), 3.91 - 3.78 (m, 1H), 3.77 - 3.50 (m, 3H), 3.45 - 3.27 (m, 8H), 3.00 - 2.84 (m, 1H), 2.78 - 2.64 (m, 2H), 2.58 - 2.39 (m, 5H), 2.38 - 2.20 (m, 2H), 2.05 - 1.78 (m, 4H), 1.77 - 1.59 (m, 2H), 1.25 (d, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.18, 170.24, 160.65, 150.75, 150.52, 142.40, 132.81, 126.94, 126.29, 116.04, 54.13, 53.60, 53.39, 52.77, 37.10, 33.79, 31.08, 28.32, 26.18, 26.05, 23.62, 18.18. HRMS (ESI-TOF) *m/*z calc'd for $C_{28}H_{40}N_5O_4$ $[M+H]^+$ 510.3080, found 510.3078.

*4.1.7.33. N-(cyclopentylmethyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**43**).* A white solid. Yield 46 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.36 - 7.20 (m, 4H), 3.89 - 3.57 (m, 3H), 3.53 - 3.31 (m, 8H), 3.26 - 3.10 (m, 1H), 3.03 - 2.85 (m, 1H),

2.76 - 2.67 (m, 2H), 2.55 - 2.20 (m, 6H), 2.09 - 1.91 (m, 1H), 1.79 - 1.41 (m, 6H), 1.29 - 1.20 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.23, 170.18, 160.61, 150.71, 150.47, 142.47, 132.72, 126.88, 126.26, 116.03, 53.57, 53.27, 52.63, 52.20, 47.38, 41.69, 38.06, 37.15, 33.75, 31.14, 30.09, 29.97, 28.31, 25.00, 24.85, 23.60. HRMS (ESI-TOF) *m/*z calc'd for $C_{29}H_{42}N_5O_4$ $[M+H]^+$ 524.3237, found 524.3237.

*4.1.7.34. N-(cyclohexylmethyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**44**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 1H), 7.25 - 7.14 (m, 4H), 3.79 - 3.49 (m, 3H), 3.43 - 3.26 (m, 8H), 3.03 - 2.92 (m, 1H), 2.90 - 2.79 (m, 1H), 2.71 - 2.56 (m, 3H), 2.45 - 2.35 (m, 2H), 2.35 - 2.15 (m, 3H), 1.70 - 1.52 (m, 5H), 1.41 - 1.28 (m, 1H), 1.20 (s, 3H), 1.18 (s, 3H), 1.14 - 1.01 (m, 3H), 0.98 - 0.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.38, 170.23, 160.65, 150.73, 150.51, 142.37, 132.78, 126.93, 126.29, 116.63, 54.07, 53.63, 53.41, 41.90, 37.18, 36.27, 33.79, 31.16, 30.49, 30.43, 28.36, 26.13, 25.56, 23.6. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{44}N_5O_4$ $[M+H]^+$ 538.3393, found 538.3385.

*4.1.7.35. N-benzyl-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**45**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 - 7.28 (m, 4H), 7.26 - 7.18 (m, 5H), 6.74 (s, 1H), 5.57 (d, J = 14.4 Hz, 1H), 3.83 (d, J = 14.5 Hz, 1H), 3.76 - 3.63 (m, 2H), 3.52 - 3.40 (m, 2H), 3.36 (s, 3H), 3.20 (s, 3H), 2.98 - 2.86 (m, 1H), 2.84 - 2.72 (m, 2H), 2.51 - 2.31 (m, 6H), 1.26 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.32, 170.39, 160.72, 150.82, 150.69, 142.97, 137.25, 133.03, 128.94, 128.59, 127.72, 127.16, 126.48, 115.10, 53.84, 53.59, 53.16, 50.92, 37.11, 33.99, 31.31, 28.51, 23.84. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{38}N_5O_4$ $[M+H]^+$ 532.2924, found 532.2921.

*4.1.7.36. N-(2-methylbenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**46**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 4H), 7.31 (s, 1H), 7.24 - 7.09 (m, 4H), 5.36 (d, J = 14.5 Hz, 1H), 4.26 (d, J = 14.5 Hz, 1H), 3.75 (s, 2H), 3.49 (s, 2H), 3.32 (s, 3H), 3.24 (s, 3H), 3.04 - 2.91 (m, 1H), 2.82 - 2.73 (m, 2H), 2.69 - 2.36 (m, 6H), 2.30 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.41, 171.06, 161.36, 151.11, 144.47, 136.87, 134.47, 132.73, 130.20, 129.75, 127.61, 127.03, 126.38, 125.64, 114.19, 53.56, 52.95, 52.41, 48.38, 36.04, 33.95, 30.83, 27.49, 23.01, 18.09. HRMS (ESI-TOF) *m/*z calc'd for $C_{31}H_{40}N_5O_4$ $[M+H]^+$ 546.3080, found 546.3078.

*4.1.7.37. N-(3-methylbenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**47**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.37 (s, 4H), 7.21 (t, J = 7.4 Hz, 1H), 7.16 - 7.03 (m, 3H), 5.34 (d, J = 14.4 Hz, 1H), 4.06 (d, J = 14.5 Hz, 1H), 3.75 (s, 2H), 3.48 (s, 2H), 3.34 (s, 3H), 3.29 (s, 3H), 3.05 - 2.93 (m, 1H), 2.82 - 2.74 (m, 2H), 2.66 - 2.38 (m, 6H), 2.33 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.56, 171.08, 161.40, 151.20, 151.12, 144.50, 138.03, 136.94, 132.73, 129.28, 128.13, 128.03, 127.01, 126.37, 125.67, 114.63, 53.51, 52.81, 52.59, 50.89, 36.08, 33.95, 30.72, 27.49, 22.97, 20.19. HRMS (ESI-TOF) *m/*z calc'd for $C_{31}H_{40}N_5O_4$ $[M+H]^+$ 546.3080, found 546.3077.

*4.1.7.38. N-(4-methylbenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**48**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.37 (s, 4H), 7.23 - 7.10 (m, 4H), 5.33 (d, J = 14.5 Hz, 1H), 4.05 (d, J = 14.4 Hz, 1H), 3.75 (s, 2H), 3.48 (s, 2H), 3.33 (s,3H), 3.29 (s, 3H), 3.09 - 2.88 (m, 1H), 2.83 - 2.72 (m, 2H), 2.66 - 2.35 (m, 6H), 2.33

(s, 3H), 1.31 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.49, 171.07, 161.39, 151.19, 151.11, 144.47, 137.11, 133.96, 132.73, 128.87, 128.64, 127.02, 126.37, 114.60, 53.54, 52.83, 52.32, 50.59, 36.10, 33.95, 30.74, 27.51, 22.99. HRMS (ESI-TOF) *m/*z calc'd for $C_{31}H_{40}N_5O_4$ $[M+H]^+$ 546.3080, found 546.3076.

*4.1.7.39. N-(3,5-dimethylbenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**49**).* A white solid. Yield 49 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.27 (m, 5H), 6.89 - 6.84 (m, 3H), 5.26 (d, J = 14.4 Hz, 1H), 3.93 (d, J = 14.4 Hz, 1H), 3.70 (s, 2H), 3.43 (s, 2H), 3.29 (s, 3H), 3.24 (s, 3H), 3.00 - 2.85 (m, 1H), 2.77 - 2.68 (m, 3H), 2.60 - 2.32 (m, 6H), 2.23 (s, 6H), 1.26 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.81, 172.38, 162.70, 152.49, 152.42, 145.82, 139.16, 138.11, 133.97, 130.13, 128.30, 127.67, 115.84, 54.77, 54.15, 53.63, 52.10, 37.37, 35.25, 31.98, 28.78, 24.26, 21.38. HRMS (ESI-TOF) *m/*z calc'd for $C_{32}H_{42}N_5O_4$ $[M+H]^+$ 560.3237, found 560.3232.

*4.1.7.40. N-(2-fluorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**50**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.47 - 7.37 (m, 1H), 7.36 - 7.34 (m, 4H), 7.30 - 7.25 (m, 1H), 7.22 - 6.99 (m, 2H), 5.17 (d, J = 14.7 Hz, 1H), 4.39 (d, J = 14.7 Hz, 1H), 3.72 (s, 2H), 3.45 (s, 2H), 3.29 (s, 6H), 3.03 – 2.87 (m, 1H), 2.73 (t, J = 7.2 Hz, 2H), 2.63 - 2.31 (m, 6H), 1.28 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 177.56, 175.08, 165.36, 165.06, 155.10, 148.47, 136.61, 135.15, 133.44, 130.90, 130.29, 128.05, 128.00, 127.54, 118.95, 118.62, 57.34, 56.70, 56.21, 48.69, 39.97, 37.89, 34.55, 31.33, 26.83. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}FN_5O_4$ $[M+H]^+$ 550.2830, found 550.2825.

*4.1.7.41. N-(3-fluorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**51**).* A white solid. Yield 46 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.42 - 7.29 (m, 5H), 7.18 - 7.09 (m, 2H), 7.07 - 6.97 (m, 1H), 5.26 (d, J = 14.9 Hz, 1H), 4.23 (d, J = 14.9 Hz, 1H), 3.76 (s, 2H), 3.48 (s, 2H), 3.34 (s, 3H), 3.31 (s, 3H), 3.04 - 2.93 (m, 1H), 2.78 (t, J = 7.2 Hz, 2H), 2.63 - 2.41 (m, 6H), 1.31 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 174.98, 172.32, 164.14, 162.64, 152.47, 152.37, 145.67, 141.22, 133.97, 131.18, 128.25, 127.62, 125.44, 116.31, 116.18, 115.22, 54.75, 54.13, 53.55, 52.14, 37.42, 35.20, 31.88, 28.80, 24.24. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}FN_5O_4$ $[M+H]^+$ 550.2830, found 550.2827.

*4.1.7.42. N-(4-fluorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**52**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.36 - 7.24 (m, 6H), 7.06 - 6.97 (m, 2H), 5.20 (d, J = 14.5 Hz, 1H), 4.14 (d, J = 14.6 Hz, 1H), 3.71 (s, 2H), 3.44 (s, 2H), 3.29 (s, 3H), 3.28 (s, 3H), 3.02 - 2.86 (m, 1H), 2.73 (t, J = 7.3 Hz, 2H), 2.60 – 2.34 (m, 6H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.82, 172.33, 163.51, 162.66, 152.48, 152.40, 145.81, 134.35, 133.95, 131.82, 128.28, 127.65, 116.14, 115.91, 54.74, 54.11, 53.52, 51.60, 37.42, 35.22, 31.90, 28.79, 24.25. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}FN_5O_4$ $[M+H]^+$ 550.2830, found 550.2828.

*4.1.7.43. N-(2-chlorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**53**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.59 - 7.53 (m, 1H), 7.47 - 7.39 (m, 5H), 7.39 - 7.33 (m, 2H), 5.38 (d, J = 15.1 Hz, 1H), 4.56 (d, J = 15.1 Hz, 1H), 3.81 (s, 2H), 3.56 (s, 2H), 3.39 (s, 3H), 3.37 (s, 3H), 3.12 - 2.97 (m, 1H), 2.91 - 2.79 (m, 2H),

2.75 - 2.45 (m, 6H), 1.37 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.75, 172.16, 162.56, 152.29, 152.24, 145.53, 135.45, 134.69, 133.92, 131.83, 130.41, 130.17, 128.23, 127.57, 115.90, 54.70, 54.02, 53.62, 49.78, 37.37, 35.11, 31.90, 28.79, 24.27. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}ClN_5O_4$ $[M+H]^+$ 566.2534, found 566.2533.

*4.1.7.44. N-(3-chlorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**54**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.40 (s, 1H), 7.37 (s, 4H), 7.33 - 7.23 (m, 3H), 5.23 (d, J = 14.8 Hz, 1H), 4.23 (d, J = 14.8 Hz, 1H), 3.76 (s, 2H), 3.50 (s, 2H), 3.34 (s, 6H), 3.05 - 2.94 (m, 1H), 2.78 (t, J = 7.1 Hz, 2H), 2.67 - 2.39 (m, 6H), 1.32 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.70, 171.03, 161.36, 151.22, 151.10, 144.45, 139.57, 133.94, 132.75, 129.75, 128.43, 127.37, 127.04, 126.84, 126.39, 114.94, 53.49, 52.85, 52.48, 50.92, 36.21, 33.96, 30.67, 27.59, 23.04. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}ClN_5O_4$ $[M+H]^+$ 566.2534, found 566.2538.

*4.1.7.45. N-(4-chlorobenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**55**).* A white solid. Yield 46 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.35 - 7.32 (m, 4H), 7.30 - 7.27 (m, 4H), 5.19 (d, J = 14.7 Hz, 1H), 4.16 (d, J = 14.7 Hz, 1H), 3.72 (s, 2H), 3.45 (s, 2H), 3.30 (d, J = 1.4 Hz, 6H), 3.03 - 2.87 (m, 1H), 2.73 (t, J = 7.2 Hz, 2H), 2.63 - 2.32 (m, 6H), 1.27 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.99, 172.46, 162.72, 152.55, 152.47, 145.83, 137.21, 134.33, 134.02, 131.46, 129.57, 128.29, 127.68, 116.09, 54.77, 54.09, 53.62, 51.85, 37.42, 35.27, 31.91, 28.78, 24.23. HRMS (ESI-TOF) *m/*z calc'd for $C_{30}H_{37}ClN_5O_4$ $[M+H]^+$ 566.2534, found 566.2532.

*4.1.7.46. N-(2-(trifluoromethyl)benzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**56**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 - 7.62 (m, 4H), 7.50 - 7.43 (m, 1H), 7.40 - 7.33 (m, 4H), 5.40 (d, J = 15.9 Hz, 1H), 4.55 (d, J = 16.0 Hz, 1H), 3.85 - 3.63 (m, 2H), 3.63 - 3.42 (m, 2H), 3.33 (s, 3H), 3.30 (s, 3H), 3.06 - 2.90 (m, 1H), 2.84 - 2.73 (m, 2H), 2.71 - 2.41 (m, 6H), 1.30 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.23, 172.25, 162.58, 152.32, 152.32, 145.62, 136.91, 133.97, 133.49, 131.04, 128.89, 128.66, 128.27, 127.61, 126.78, 125.64, 116.13, 79.45, 54.73, 54.11, 53.60, 43.09, 37.39, 35.16, 31.85, 28.77, 24.26. HRMS (ESI-TOF) *m/*z calc'd for $C_{31}H_{37}F_3N_5O_4$ $[M+H]^+$ 600.2798, found 600.2795.

*4.1.7.47. N-(3-(trifluoromethyl)benzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**57**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 - 7.61 (m, 1H), 7.60 - 7.42 (m, 4H), 7.35 - 7.29 (m, 4H), 5.16 (d, J = 14.8 Hz, 1H), 4.36 (d, J = 14.8 Hz, 1H), 3.71 (s, 2H), 3.43 (s, 2H), 3.29 (d, J = 2.6 Hz, 6H), 3.01 - 2.85 (m, 1H), 2.76 - 2.60 (m, 2H), 2.64 - 2.32 (m, 6H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.79, 171.09, 161.41, 151.24, 151.14, 144.58, 138.48, 132.69, 132.26, 130.27, 129.00, 127.00, 126.37, 125.28, 124.25, 123.98, 114.91, 53.40, 52.88, 52.35, 51.14, 36.13, 33.96, 30.59, 27.49, 22.96. HRMS (ESI-TOF) *m/*z calc'd for $C_{31}H_{37}F_3N_5O_4$ $[M+H]^+$ 600.2798, found 600.2787.

*4.1.7.48. N-(2-methoxybenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**58**).* A white solid. Yield 47 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.40 - 7.37 (m, 4H), 7.34 - 7.27 (m, 2H), 6.99 - 6.91 (m, 2H), 5.16 (d, J = 14.3 Hz, 1H), 4.39 (d, J =

14.2 Hz, 1H), 3.87 - 3.65 (m, 5H), 3.62 - 3.40 (m, 2H), 3.34 (s, 3H), 3.29 (s, 3H), 3.05 - 2.92 (m, 1H), 2.83 - 2.72 (m, 2H), 2.64 - 2.38 (m, 6H), 1.32 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.80, 172.37, 162.86, 159.00, 152.50, 152.42, 145.40, 134.04, 131.85, 130.37, 128.32, 127.67, 125.70, 121.58, 116.10, 111.47, 55.75, 54.82, 54.08, 53.61, 47.14, 37.32, 35.24, 32.12, 28.74, 24.27. HRMS (ESI-TOF) *m/z* calc'd for $C_{31}H_{40}F_3N_5O_5$ $[M+H]^+$ 562.3029, found 562.3024.

*4.1.7.49. N-(3-methoxybenzyl)-N-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(4-(4-isopropylbenzoyl)piperazin-1-yl)propanamide (**59**).* A white solid. Yield 48 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.35 - 7.28 (m, 4H), 7.24 - 7.12 (m, 1H), 6.85 - 6.77 (m, 3H), 5.24 (d, J = 14.6 Hz, 1H), 4.05 (d, J = 14.6 Hz, 1H), 3.72 (m, 5H), 3.45 (s, 2H), 3.28 (s, 3H), 3.24 (s, 3H), 2.98 - 2.87 (m, 1H), 2.79 - 2.66 (m, 2H), 2.59 - 2.33 (m, 6H), 1.25 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.94, 172.40, 162.72, 161.23, 152.50, 152.44, 145.72, 139.84, 133.98, 130.55, 128.30, 127.68, 121.98, 116.01, 115.38, 114.00, 55.66, 54.80, 54.13, 53.64, 52.30, 37.41, 35.26, 31.96, 28.79, 24.25. HRMS (ESI-TOF) *m/z* calc'd for $C_{31}H_{40}F_3N_5O_5$ $[M+H]^+$ 562.3029, found 562.3026.

4.2. Biological evaluation

4.2.1. In vitro ALDH enzymatic activity assays.

Human ALDH1A1, Human ALDH1A2, ALDH1A3, and ALDH3A1 were purchased from Novoprotein (Shanghai, China). Human ALDH2 was purchased from Abcam (Cambridge, MA).

Inhibition of synthesized 1,3-dimethylpyrimidine-2,4-diones and respective IC_{50} curves were determined by measuring the formation of NAD(P)H spectrophotometrically at 340 nm on the multiscan spectrum using purified

recombinant ALDH1A1, ALDH1A2, ALDH1A3, ALDH2 and ALDH3A1 [19]. All reactions were initiated by adding the substrate of propionaldehyde or benzaldehyde after a 15 min preincubation (25 °C, protected from light) period. For ALDH1A family members and ALDH2, reactions contained 100~200 nM enzyme, 500 μM NAD^+ and 400 μM propionaldehyde in 20 mM Tris-HCl, pH 7.5 at 25 °C. The assay for ALDH3A1 activity used 20 nM enzyme, 300 μM $NADP^+$ and 300 μM benzaldehyde. Inhibition was initially tested using 10 μM compounds and respective IC_{50} curves for propionaldehyde oxidation were calculated by varying the concentration of the compounds from 0 to 200 μM. The change of fluorescence intensity over 10-15 min reaction period was normalized no-inhibitor and no-enzyme controls. *N*,*N*-diethylaminobenzaldehyde (DEAB) was used as internal standard with comparable activity to reported potency [20].

4.2.2. Cell viability studies.

The HepG2 cells were obtained from American Type Culture Collection. Cells were cultured in DMEM containing 10 % FBS, 1 % non-essential amino acids, antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin), 2 mM L-glutamine, and 3.7 g/L $NaHCO_3$ in a humidified incubator of 5 % CO_2 and 95 % air atmosphere at 37 °C.

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was performed to evaluate to cytotoxic effects of compounds **1**, **46**, **50**, **53**, **56** and **57** on HepG2 cells. HepG2 cells were plated into 24-well plates at a density of 2.5 × 10^5 cells/well. When growing to 70% confluence, the HepG2 cells were incubated with culture medium containing different levels of compounds (0.1, 1 and 10 μM) for 24 h. Then 500 μL MTT was added to 24-well plates. After cultured for 4 h, 500 μL

DMSO was added and the mixture was shaked for 10 min at 37 °C. The absorbance of the mixture was determined at 490 nm on the multiscan spectrum.

4.2.3. In vitro study on glucose consumption in HepG2 cells.

Effects of compounds **1**, **46**, **50**, **53**, **56**, **57** and **Metformin** on glucose consumption in HepG2 cells were investigated. HepG2 cells were plated into 24-well plates at a density of 2.5×10^5 cells/well. When growing to 70% confluence, the HepG2 cells were incubated with culture medium containing different levels of compounds (1000 μM for **metformin**, 1 and 10 μM for compounds **1**, **46**, **50**, **53**, **56**, **57**) for 24 h, the treated HepG2 cells were collected to assess glucose consumption [18]. Glucose consumption assay in HepG2 cells was measured according to method previously described [21]. Protein concentrations of cells were measured using BSA protein assay kits.

4.2.4. Metabolic stability assay in human liver microsomal.

The metabolic stability assay in human liver microsomal was determined by measuring the percentage of compound remaining after incubation. The assay incubation system contained 0.5 mg/ mL microsomal protein, 2 μg/ mL compound concentration and NADPH regeneration system (containing 0.5 mM $NADP^+$, 10 mM glucose 6- phosphate, 5 mM $MgCl_2$ and 1 unit/mL G6PDH) in 100 mM phosphate buffer at pH 7.4. The DMSO concentration was less than 0.1% in the final incubation system. The reaction was initiated by adding NADPH regeneration system after a 5 min preincubation period at 37°C. At 0, 5, 10, 15, 30 and 45 min of incubation, 15 μL of reaction mixture were taken out and the reaction was quenched by adding 5-fold cold acetonitrile containing 2 μg /mL theophylline as internal standard for quantification.

After a 10 min centrifugation at 10000 rpm, supernatants were collected for LC-MS/MS analysis to determine the amount of compound remaining.

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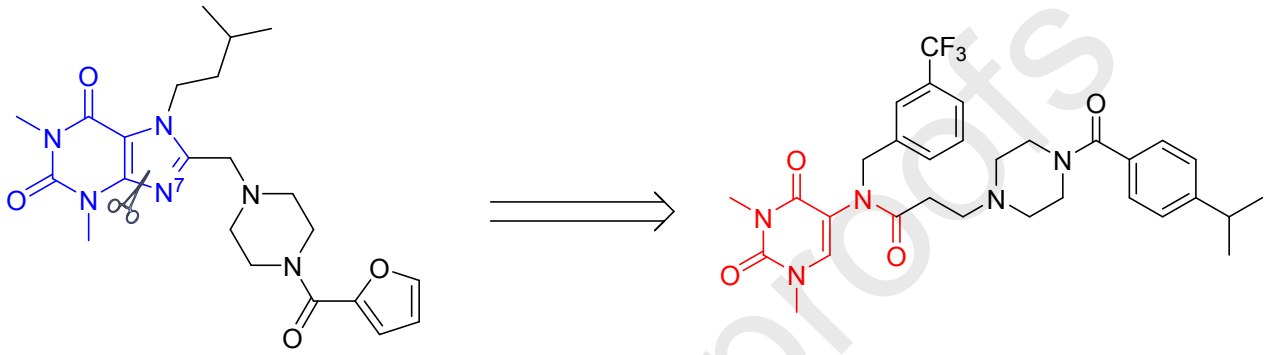
Highlights

1. A series of 1,3-dimethylpyrimidine-2,4-diones were identified as potent ALDH1A1 inhibitors.
2. Several tested compounds demonstrated high selectivity to ALDH1A1 against other ALDH isozymes.
3. Compound **57** can effectively improve glucose consumption in HepG2 cells comparing to compound **1**.

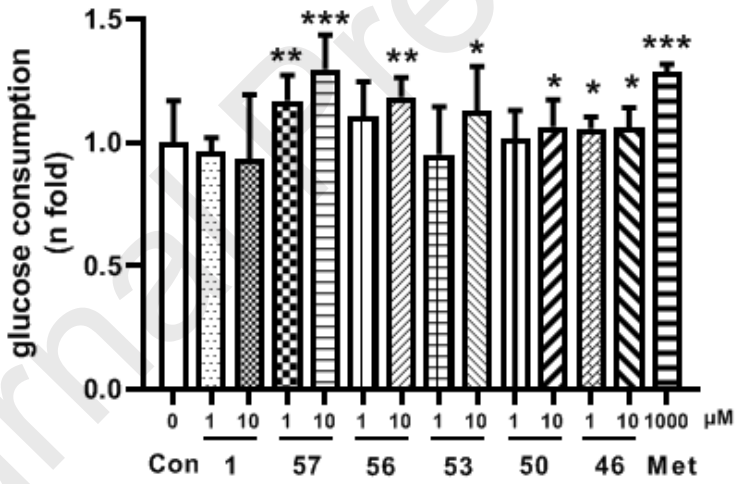
Graphical Abstract

Design, synthesis of 1,3-dimethylpyrimidine-2,4-diones as potent and selective aldehyde dehydrogenase 1A1 inhibitors with glucose consumption improving activity



CM26 (**1**): ALDH1A1 IC_{50} = 1.21 ± 0.18 μM

57: ALDH1A1 IC_{50} = 0.379 ± 0.07 μM



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: