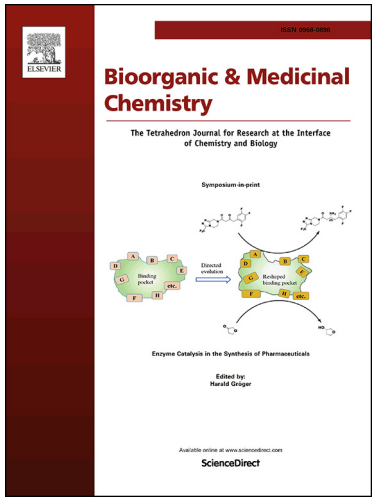
Accepted Manuscript



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Design and synthesis of novel 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide derivatives as potent Mnks inhibitors by fragment-based drug design

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Abstract

A novel series of 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide derivatives featured with various C-2 substituents were designed and synthesized as Mnks inhibitors through fragment-based drug design. Among them, **5b**, **5i**, **5o** and **8k** showed the best Mnk2 inhibitory activity with IC_{50} values of 1.45, 1.16, 3.55 and 0.27 μM, respectively. And these compounds inhibited the activity of Mnk1 at the same time. Furthermore, compounds **5o** and **8k** exhibited anti-proliferative effects to human leukemia cancer THP-1 and MOLM-13 cell lines and colon cancer HCT-116 cell line. Moreover, western blot assay suggested that **8k** could decrease the levels of p-eIF4E in a dose-dependent manner in HCT-116 cells. Docking studies demonstrated strong interactions between **8k** and Mnk2. Therefore, this unique benzofuran scaffold demonstrated great potential to be further explored as potent Mnks inhibitors with improved potency.

Keywords: 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide derivatives, fragment-based drug design, anti-proliferative effects, Mnks inhibitors.

1. Introduction

Dys-regulation of protein synthesis is implicated in the progression of various pathologies, most notably cancer.[1] As a key rate-limiting step in protein synthesis, initiation of cap-dependent translation is contingent on the availability and activity of eukaryotic initiation factor 4E (eIF4E).[2] eIF4E, the most prominent substrate of mitogen-activated protein kinase (MAPK)-interacting kinases (Mnks), is phosphorylated exclusively by Mnks at the conserved serine 209.[3] Mnks comprise a subfamily of Ser/Thr kinases and belong to the group of Ca^{2+}/calmodulin-dependent kinases. Mnks are present in two isoforms: Mnk1 and Mnk2, the catalytic domains of Mnk1/2 are highly homologous with ~80% sequence identity between them. Despite this, Mnk2 possesses high constitutive basal activity comparing to Mnk1, which has been shown in *in vitro* studies.[4] Though the activity of Mnks is necessary for eIF4E-mediated tumorigenesis, it is dispensable for normal tissue development, which has been proved by the experiments of silencing Mnk1 with small hairpin RNA (shRNA) or knocking out Mnk1/2.[5] Thus, inhibiting Mnks offers an exciting opportunity for effectively treating cancer with low toxicity.

Despite the fact that Mnks are potential targets for cancer therapy with better outcomes and fewer side effects, the role of Mnk1/2 in cancer still remains elusive due to the absence of potent and selective probes. In many instances, hypotheses have been built upon unspecific Mnk1/2 inhibitors such as CGP-57380 or cercosporamide. Lately, the first two clinical programs targeting Mnks in oncology have been revealed (eFT508 and BAY1143269, the structure of BAY1143269 has not been disclosed).[6]

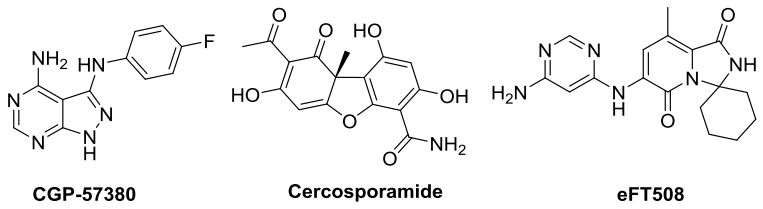


Figure 1. Chemical structures of known Mnks inhibitors.

ATP-competitive kinase inhibitors typically form 1~3 hydrogen bonds with the hinge region of the kinase, aryl carbamoyl became one of new fragments binding to the hinge region of kinases appeared in the structure of many candidate compounds. For instance, the VEGFR inhibitor lenvatinib

approved for advanced thyroid carcinoma at 2015 showed the potential advantages of the fragment of aryl carbamoyl (**Figure 2**).[7-14]

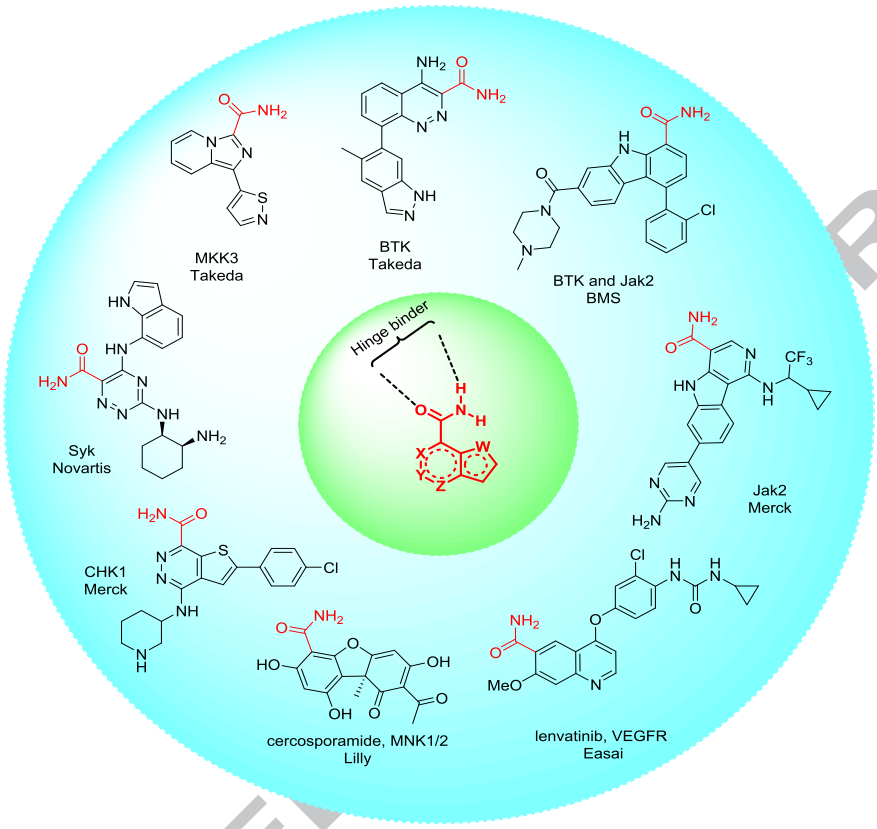
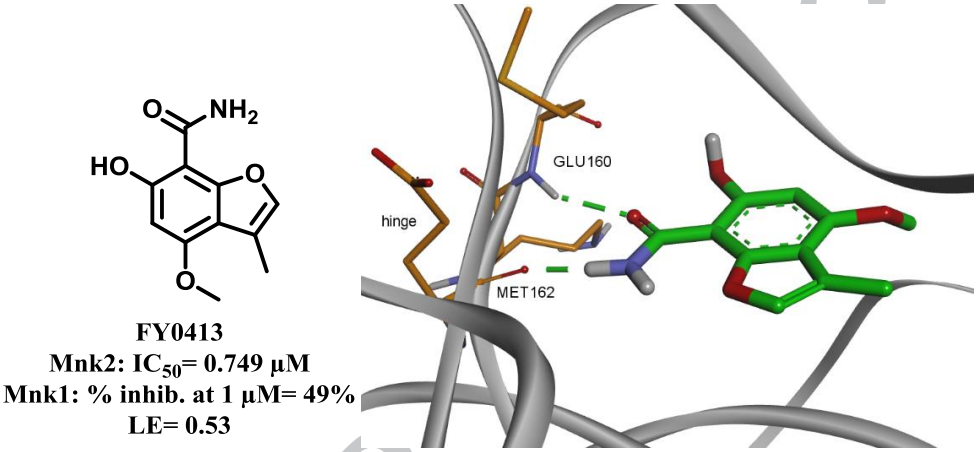


Figure 2. Kinase inhibitors in development with carbamoyl moiety as hinge binder.

The carbamoyl group could form two hydrogen bonds with the hinge region of Mnks via carbonyl and NH_2. Ideally, introducing a hydroxyl group to *ortho* position of carbamoyl could form another hydrogen bond with the hinge. In our efforts to discover novel Mnks inhibitors, bioactive benzofuran scaffold was chosen as the mimics of adenine core, and introduces 6-hydroxy and 7-carbamoyl, a hit compound of 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide (**FY0413**) was prepared as a potential Mnks inhibitor. **FY0413** showed an IC_{50} of 0.749 μM for Mnk2 and 49% inhibition ratio for Mnk1 at 1 μM, the ligand efficiency of **FY0413** is 0.53. Besides, **FY0413** showed moderate anti-proliferative activity to leukemia and colon cancer cells (IC_{50} THP-1: 32.35 μM, MOLM-13: 24.81 μM, HCT-116: 26.05 μM) *in vitro*. The docking mode (**Figure 3**) of **FY0413** with Mnk2 (PDB: 2HW7) indicated that only two hydrogen bonds were formed between **FY0413** and Mnk2. Besides, there is still a lot of space in the ATP-binding pocket, introducing a substituent at C-2 position of **FY0413** may occupy it with additional interactions and result better potency. To illuminate the Mnks inhibitory

activity by this unique and proprietary scaffold in a systemic way, a series of novel 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide derivatives featured with various C-2 substituents were described. Their inhibitory activities toward Mnks as well as the *in vitro* anti-proliferative activity toward two leukemia cell lines (THP-1, MOLM-13) and one colon cancer cell line (HCT-116) were studied. Finally, the inhibition toward the phosphorylation of eIF4E was also investigated with representative compound in HCT-116 cells. The proposed binding mode in the ATP pocket of Mnk2 was illustrated.



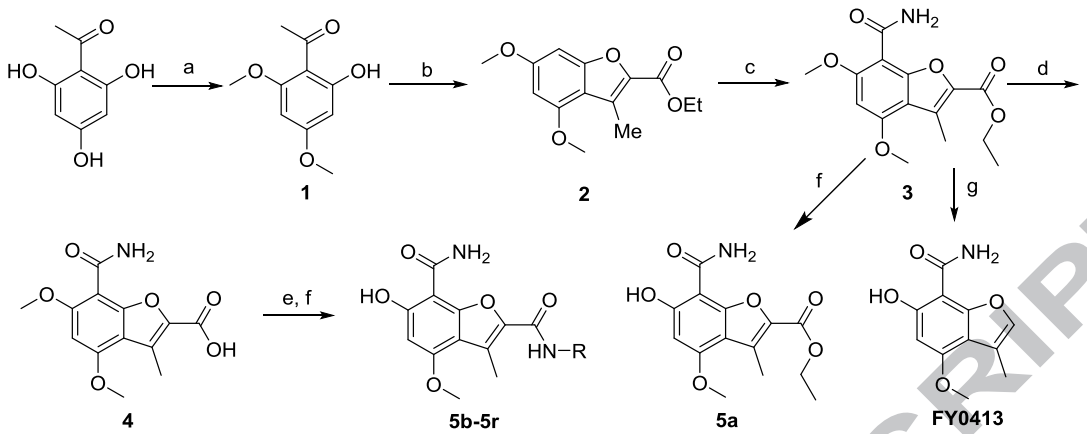
FY0413
Mnk2: IC_{50}= 0.749 μM
Mnk1: % inhib. at 1 μM= 49%
LE= 0.53

Figure 3. The structure of **FY0413** and the docking mode of **FY0413** in the ATP binding pocket of the Mnk2 kinase domain (**2HW7** in PDB). H-Bonding interactions were presented with green line.

2. Results and discussion

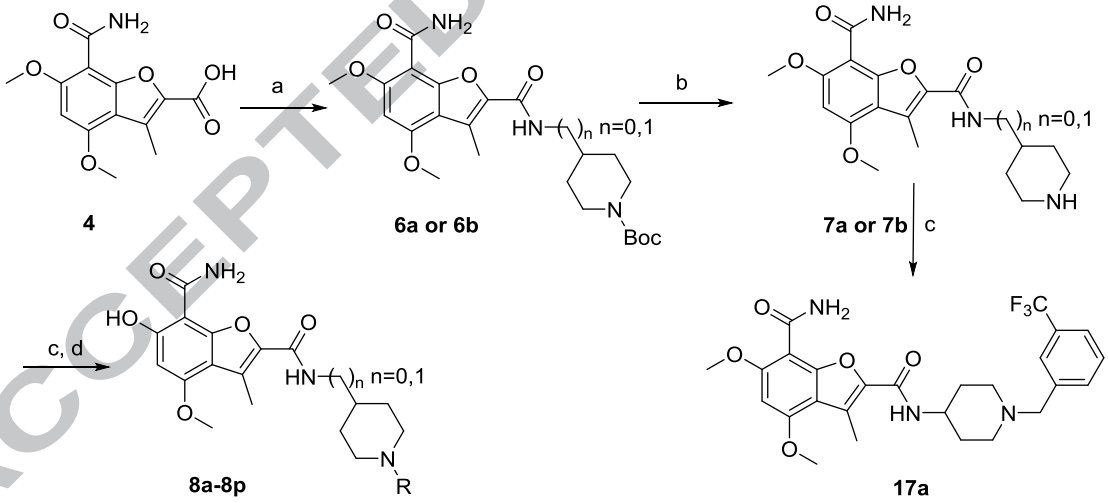
2.1. Chemistry

The synthesis of compounds **5a-5r** and **FY0413** was described in **Scheme 1**. Commercial starting material 1-(2,4,6-trihydroxyphenyl)ethan-1-one was reacted with two equivalents saturated dimethylsulfate to afford dimethylated intermediate **1**, the key intermediate **2** was obtained by condensation and cyclization with ethyl bromoacetate. Introducing carbamoyl at the 7-position of **2** with CSI to afford the intermediate ethyl 7-carbamoyl-4,6-dimethoxy-3-methylbenzofuran-2-carboxylate **3**. Followed by ester hydrolysis and reaction of the resulted acid with amino to yield the corresponding amide, treatment of the resulted amide with BBr_3 afforded **5b-5r**. Treatment of **3** with either 30% hydrobromic acid or BBr_3 afforded the hit compound **FY0413** and **5a**.



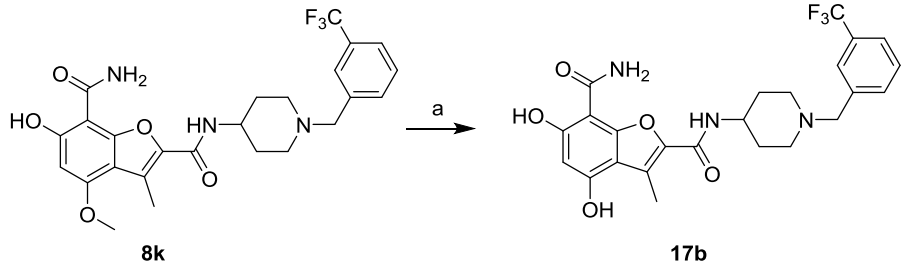
Scheme 1. Synthesis of **5a-5r** and **FY0413**. Reagents and Conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone; (b) $BrCH_2COOC_2H_5$, K_2CO_3, DMF, 70-90°C; (c) CSI, CH_3CN, 0°C to r.t., then 1N HCl; (d) NaOH, CH_3OH/H_2O, 40°C; (e) RNH_2, HATU, DIEA, DMSO, r.t.; (f) BBr_3, CH_2Cl_2, -40°C to r.t.; (g) HBr(30%), CH_3COOH, reflux.

The synthesis of compounds **8a-8p** and **17a** was described in **Scheme 2**. The intermediate **4** reacted with 4-amino-1-Boc-piperidine or 4-aminomethyl-1-Boc-piperidine, then deprotection of Boc group afforded **7**, **7** reacted with substituted benzyl chloride or substituted benzoyl chloride, then deprotection of methyl group afforded **8a-8p**. **7a** reacted with 3-trifluoromethyl benzyl chloride to afford **17a**.



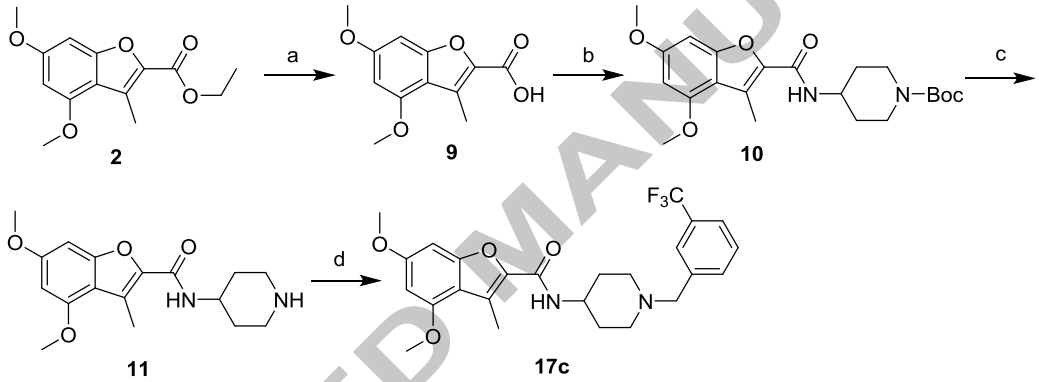
Scheme 2. Synthesis of **8a-8p** and **17a**. Reagents and Conditions: (a) 4-amino-1-Boc-piperidine or 4-aminomethyl-1-Boc-piperidine, HATU, DIEA, DMSO, r.t.; (b) HCl in ethyl acetate, r.t.; (c) substituted benzyl chloride or benzoyl chloride, K_2CO_3, DMF, 80°C; (d) BBr_3, CH_2Cl_2, -40°C to r.t..

Treatment of **8k** with BBr_3 afforded **17b**, which was described in **Scheme 3**.



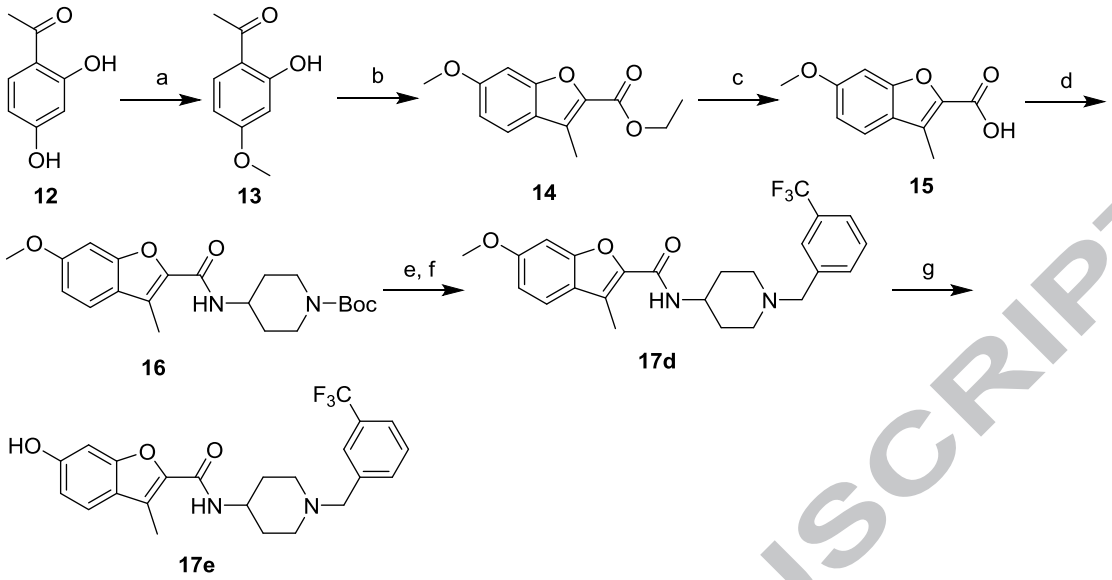
Scheme 3. Synthesis of **17b.** Reagents and Conditions: (a) BBr_3, CH_2Cl_2, -40°C to r.t..

The synthesis of **17c** was described in **Scheme 4**. The intermediate **9** was synthesized by ester hydrolysis of **2**. Treatment of **9** with 1-Boc-piperidine-4-amino, deprotection of Boc group and reacted with 3-triflurомethylbenzyl bromide to afford **17c**.



Scheme 4. Synthesis of **17c**. Reagents and Conditions: (a) NaOH, H_2O/CH_3OH, reflux; (b) 4-amino-1-Boc-piperidine, HATU, DIEA, DMSO, r.t.; (c) HCl in ethyl acetate, r.t.; (d) 3-triflurомethylbenzyl bromide, Cs_2CO_3, DMF, 80°C.

The synthesis of compounds **17d** and **17e** were described in **Scheme 5**. Selecting 1-(2,4-dihydroxyphenyl)ethanone (**12**) as starting material, the 4-hydroxy of **12** was protected selectively by methyl. The synthesis of **15** was conducted through cyclization and ester hydrolysis of **13**. Scaffold **15** was further reacted with 1-Boc-piperidine-4-amino to yield **16**, deprotection the Boc group and then reacted with 3-triflurомethylbenzyl bromide to afford **17d**. Treatment of **17d** with BBr_3 afforded **17e**.



Scheme 5. Synthesis of **17d** and **17e**. Reagents and Conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone; (b) $BrCH_2COOC_2H_5$, K_2CO_3, DMF, 70-90°C; (c) NaOH, CH_3OH/H_2O, 40°C; (d) RNH_2, HATU, DIEA, DMSO, r.t.; (e) HCl in ethyl acetate, r.t.; (f) 3-trifluromethylbenzyl bromide, Cs_2CO_3, DMF. 80°C; (g) BBr_3, CH_2Cl_2, -40°C to r.t..

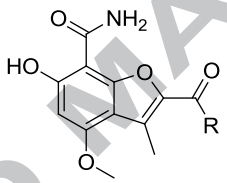
2.2. Biological activity

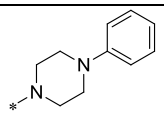
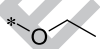
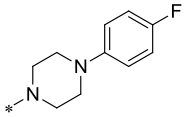
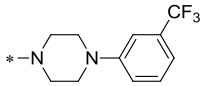
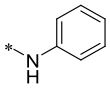
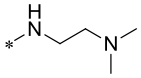
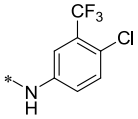
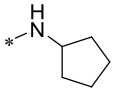
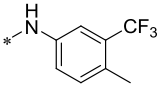
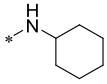
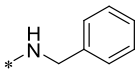
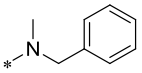
At the initial stage of the study, only the inhibition ratio at 10 μM and 1 μM against Mnk2 were determined for each compound with an advanced HTRF method at *Cisbio*. **CGP-57380** exhibited inhibitory effect with IC_{50} value of 1.53 μM in the tested system (the reported value is 1.60 μM), and the results for all target compounds were shown in **Table 1** ~ **Table3**. The results indicated that portion of the target compounds exhibited potent activities towards Mnk2. Among which, compounds **5b**, **5i**, **5l**, **5o** and **8k** displayed a inhibition ratio > 50% at 10 μM; compounds **5a**, **5c**, **5n**, **8a**, **8c**, **8e**, **8g**, **8h**, **8i**, **8l**, and **8p** displayed a inhibition ratio > 30% at 10 μM. **5a** with ethyl ester substituted at C-2 position exhibited little potency (41% at 10 μM, inhibition ratio at 10 μM was chosen to analysis the SAR below); *N*-methylformamide substituent (**5b**) led to elevated inhibitory activity, indicating that amide bond is favorable at C-2 position than ester bond. So the followed compounds employed amide bond as linker, and a more systematic study of the substituents on nitrogen atoms were carried out.

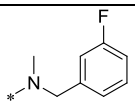
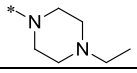
The potency of **5c** with *N*, *N*-dimethyl substituted was inferior to **5b**, indicated that the presence of hydrogen atom at amide of C-2 is essential for the activity. Prolonged the substituent (**5d**) resulting the decrease of potency, indicating that the larger flexible chain system was not suitable for the kinase pocket. **5e** and **5f** with cyclopentyl and cyclohexyl substituted showed little potency. Contracted the exo nitrogen atom of amide into an endo one resulted **5g-5i**. **5g** and **5h** exhibited little potency to Mnk2,

but **5i** with *N*-ethylpiperazinyl substituted was found to be more potent than **5g** and **5h**, which indicated that piperazinyl is helpful to the inhibitory activity. The substituted phenyl was introduced to the 4-position of piperazinyl, subsequently. The results indicated that the substituents on phenyl influence the potency by the trends: 3-CF_3 (**5l**: 61%) > H (**5j**: 15%) > 4-F (**5k**: 10%). **5m-5r** showed the effect of aromatic amine substituents on activities. **5m** without substituent on phenyl lost potency completely. However, when the substituents were introduced into the 3- and 4-position of phenyl, its potencies were greatly improved (**5n** and **5o**). Among them, **5o** with 4-methyl-3-trifluoromethyl substituents on phenyl exhibited better activity. Replacing the phenyl by benzyl (**5p**) resulted improved activity, and when the hydrogen atom on amide was substituted by methyl (**5q**), the activity lost completely, which was similar to **5b**.

Table 1. The structure and inhibitory activities of compounds **FY0413** and **5a-5r** on Mnk2 kinase.



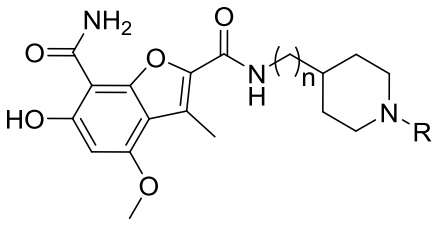
Compd.	R	Inhibition ratio/%[a]		Compd.	R	Inhibitionratio/%[a]	
		10 μM	1 μM			10 μM	1 μM
FY0413	-	77	43	**5j**		15	8
5a		41	21	**5k**		10	5
5b		73	66	**5l**		61	28
5c		45	24	**5m**		0	0
5d		24	19	**5n**		35	18
5e		22	6	**5o**		57	75
5f		28	20	**5p**		28	24
5g		24	12	**5q**		0	0

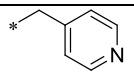
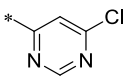
5h		23	19	**5r**		23	11
5i		79	36				

[a] All results represent the mean of duplicate experiments.

5i with *N*-ethylpiperazinyl substituted exhibited best inhibitory activity in the above optimization. Considering the importance of hydrogen atom on the amide, piperazinyl was replaced with 4-aminepiperidinyl and ethyl was replaced with lager substituents such as pyridyl, substituted pyrimidyl, substituted benzoyl or substituted benzyl, which afforded **8a-8p**. The inhibitory activity indicated that aromatic heterocyclic substituents on piperidinyl had little effect on enzyme binding (**8a**, **8b**), so the following compounds were designed to substituted benzyl. The inhibitory activity indicated that the substituents on phenyl influenced the activity by the trends: 3-CF_3 >> 4-Cl ≈ 4-CF_3 ≈ 4-CH_3 > 2-F > 4-F (**8k** >> **8h** ≈ **8i** ≈ **8g** > **8c** > **8d**). **8k** with 3-trifluoromethyl substituted exhibited the best inhibitory activity on Mnk2, which is similar to **5l** and **5o**. Replacing the substituted benzyl with substituted benzoyl decreased the kinase inhibitory activity (**8f** < **8e**, **8j** < **8i**). The inhibitory activity of di-substituted compounds was weaker than mono-substituted compounds, for example, **8m ~ 8p** < **8h**. The above results showed that the type of substituents is more important than the number of substituents, and 3-trifluoromethyl substituted (**8k**) is the best.

Table 2. The structure and inhibitory activities of compounds **8a-8p** on Mnk2.



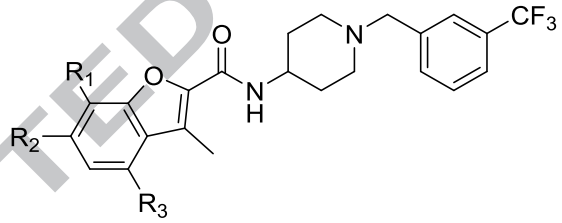
Compd.	R	n	Inhibition ratio/%[a]		Compd.	R	n	Inhibitionratio/%[a]	
			10 μM	1 μM				10 μM	1 μM
8a		0	32	15	**8i**	4-CF_3-benzyl	0	43	24
8b		0	11	0	**8j**	4-CF_3- benzoyl	0	29	26
8c	2-F-benzyl	0	31	29	**8k**	3-CF_3-benzyl	0	90	85
8d	4-F-benzyl	0	15	8	**8l**	3-CF_3-benzyl	1	49	23
8e	4-F-benzyl	1	41	32	**8m**	2,6-diCl-benzyl	0	27	1
8f	4-F-benzoyl	1	23	16	**8n**	2,4-diCl-benzyl	0	0	0
8g	4-CH_3-benzyl	0	40	27	**8o**	2,3-diCl-benzyl	0	21	18

8h	4-Cl-benzyl	0	45	29	**8p**	3,4-diCl-benzyl	1	38	21

[a] All results represent the mean of duplicate experiments.

To prove the idea of the *ortho* hydroxyl carboxamide substituents is beneficial to the binding, the substituents on C-2 was fixed and compounds **17a-17e** were designed to discuss the optimal substituents on position C-4, C-6 and C-7(**Table 3**). The inhibitory activities indicated that compound with 6-hydroxy-7-carboxamide substituents (**8k**) exhibited best Mnk2 inhibitory activity, removing the carbamoyl on position C-7 (**17c-17e**) or replacing hydroxyl to methoxy on position C-6 (**17a**) sharply decreased the kinase inhibitory activity, which indicated the significance of 6-hydroxy-7-carbamoyl substituents for inhibitory activity. Meanwhile, replacement the methoxy on position C-4 to hydroxyl led to depressed activity, most strikingly elucidated by **17b**, which indicated that 4-methoxy substituent is essential to Mnk2 inhibitory activity. All these data confirmed that the substituents on hit compound (**FY0413**) are necessary for Mnk2 inhibitory activity, and this rule is appropriate for compounds with substituents on C-2.

Table 3. The structure and inhibitory activities of compounds **8k** and **17a-17e** on Mnk2.



Compd.	R_1	R_2	R_3	Inhibition ratio/%[a]	
				10 μM	1 μM
8k	carbamoyl	hydroxy	methoxy	90	85
17a	carbamoyl	methoxy	methoxy	29	2
17b	carbamoyl	hydroxy	hydroxy	34	1
17c	H	methoxy	methoxy	35	6
17d	H	methoxy	H	27	0
17e	H	H	H	28	0

[a] All results represent the mean of duplicate experiments.

Compounds **5b**, **5i**, **5o** and **8k** showed potent inhibitory activity against Mnk2, the IC_{50} values against Mnk2 and the inhibition ratio at 10 μM, 1 μM and 0.1 μM against Mnk1 were determined. Meanwhile, the anti-proliferative activity of these compounds against THP-1, MOLM-13 and HCT-116 cell lines was further evaluated using MTT assay. The results fitted well with the Mnk2 inhibition data. **5b**, **5i**, **5o** and **8k** exhibited potent Mnk2 inhibitory activity with the IC_{50} values of 1.45, 1.16, 3.55 and

0.27 μM, respectively, which demonstrated Mnk1 inhibition at the same time (inhibition ratio at 10 μM: 39%, 30%, 23% and 59%, respectively). **8k** showed a three-fold potency improvement compared with **FY0413**, similar to pan-kinases inhibitor sorafinib, and it was more potent than CGP-57380, a widely used Mnks inhibitor. The anti-proliferative activity of **8k** is superior to CGP-57380.

Table 4. Inhibition effects of selected compounds towards Mnks and cancer cells.

Compd.	Mnk1 inhibition ratio/%[a]			IC_{50}/μM			
	10 μM	1 μM	0.1 μM	Mnk2[a]	THP-1[b]	MOLM-13[b]	HCT-116[b]
FY0413	39	49	34	0.749	32.35	24.81	26.05
5b	39	26	11	1.45	>50	n.d.	n.d.
5i	30	7	3	1.16	18.76	13.54	15.72
5o	23	23	12	3.55	>50	n.d.	n.d.
8k	59	41	20	0.273	6.30	3.15	2.77
Sorafinib	n.d.[c]			0.262	n.d.	n.d.	n.d.
CGP-57380[c]	IC_{50}=0.85 μM[d]			1.53	11.68	8.24	10.21

[a] All results represent the mean of duplicate experiments.

[b] Data shown are means of three independent experiments.

[c] Not determined;

[d] CGP-57380 was demonstrated as IC_{50} values towards Mnk1.

To gain further insights into the mechanisms of action of these compounds, compound **8k**, which possessed significant Mnks and cellular inhibitory effects, was selected to examine the inhibitory effect on the phosphorylation level of eIF4E at Ser209 with western bolt analysis in HCT-116 cells. eIF4E, the most prominent substrate of mitogen-activated protein kinase (MAPK)-interacting kinases (Mnks), is phosphorylated exclusively by Mnks at the conserved serine 209, thus the phosphorylation level of eIF4E could reflect the residual activity of Mnks indirectly. HCT-116 cells were treated with **8k** at different concentrations or DMSO as negative control for 24 h (**Figure 4**). The results indicated that treatment with **8k** decreasing the levels of phosphorylation of eIF4E at Ser209, but barely affected the levels of eIF4E, confirming their cellular Mnks inhibitory activity.

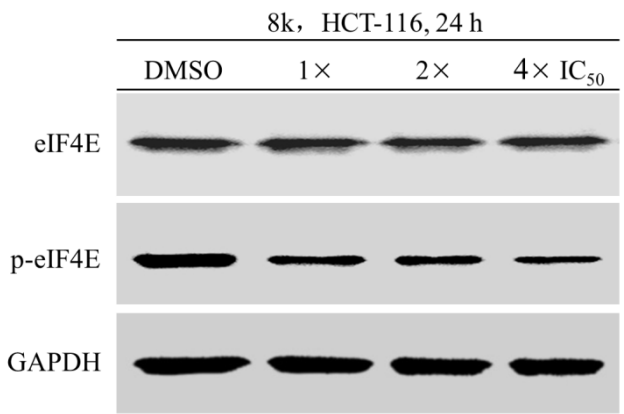


Figure 4. **8k** decreased the expression of p-eIF4E in HCT-116 cells.

In order to understand the binding mode of **8k** with Mnk2 kinase, the docking experiments were conducted with Discovery studio software based on the X-ray co-crystal structure of Mnk2 with staurosporine (PDB: 2HW7). Supporting the docking results, staurosporine was first docked and fitted well with that in crystal structure. Then **8k** was docked with the same parameters. The binding mode (**Figure 5**) demonstrated strong interactions between **8k** and Mnk2. Three hydrogen bonds were established between hydroxyl and carbamoyl and Glu160 and Met162 as expected. Besides, the carbonyl of amide formed a unique hydrogen bond with Ser166. Apart from hydrogen bonding, the benzofuran scaffold and 3-trifluoromethylbenzyl showed hydrophobic and van der Waals interactions with residues Val98, Ala111, Leu168 and Leu212 of the receptor protein, which likely provided a significant contribution to Mnk2 binding and inhibition.

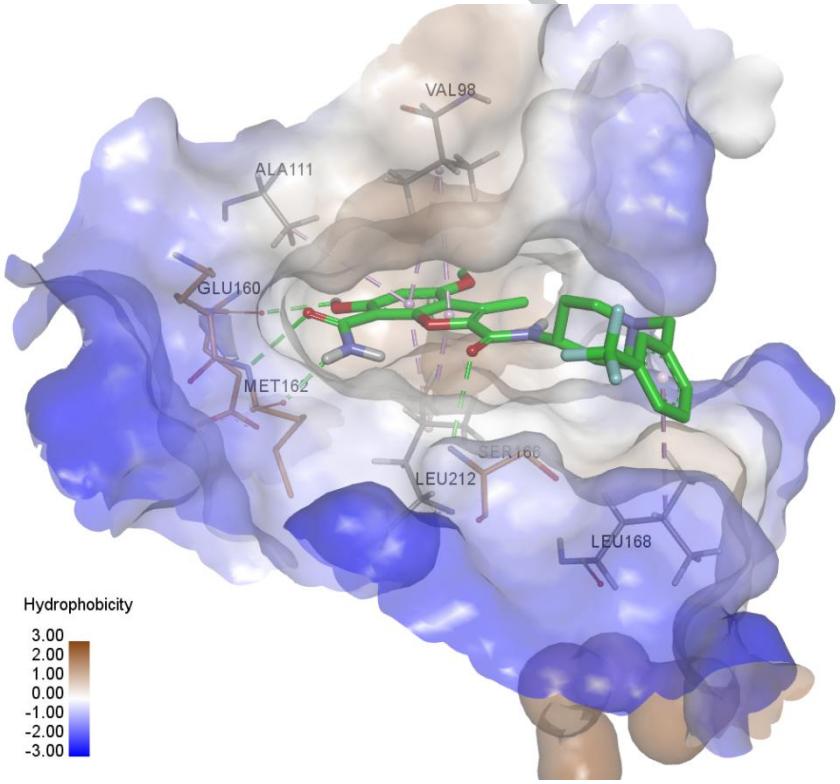


Figure 5. The proposed binding mode of **8k** in the ATP binding pocket of the Mnk2 kinase domain (**2HW7** in PDB). H-Bonding interactions were presented with green line.

3. Conclusion

We report herein the discovery and synthesis of novel benzofuran derivatives as potent Mnks inhibitors. Among the 39 compounds, **5b**, **5i**, **5o** and **8k** were the most potent Mnk2 inhibitor with IC_{50}

values of 1.45, 1.16, 3.55 and 0.27 μM, respectively. And these compounds inhibited the activity of Mnk1 at the same time. **5o** and **8k** exhibited low micromolar anti-proliferative activity toward leukemia cancer THP-1, MOLM-13 and colon cancer HCT-116 cell lines. In addition, **8k** could block the phosphorylation of eIF4E in a dose-dependent manner in HCT-116 cells. Molecular modeling illustrated a strong binding effect of **8k** within Mnk2 ATP-binding site. Together, these results provide compelling evidence that the anti-proliferative activity of these compounds in THP-1, MOLM-13 and HCT-116 cell lines was mediated, at least in part, by the inhibition of Mnks. Therefore, this unique benzofuran scaffold demonstrated great potential to be further explored as potent Mnks inhibitors with improved potency. Efforts are ongoing to further optimize and provide a systematic structure-activity analysis at the proper position and the results will be reported in due course.

4. Experimental

4.1. Chemistry

Melting point was measured with X-4 digital micro melting point apparatus with temperature unrevised. Infra-red (IR) spectra were recorded on Bruker IR-27G spectrometer with KBr pellets. ^{1}H-NMR spectra were measured with a Bruker ARX (400 MHz) spectrometer and ^{13}C-NMR with a Bruker AV (100 MHz) instrument. Chemical shifts are recorded in δ units using tetramethylsilane as the standard (NMR peak description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak). Low resolution mass spectrometry (MS) were recorded with an Agilent 1100 ion trap liquid chromatography-mass spectrometer. Organic solutions were dried over anhydrous $MgSO_4$ during workup. Column chromatography was carried out on TELEDYNE ISCO Combiflash Rf+ preparative liquid chromatograph. Silica gel 60 (200-300 mesh) and TLC were purchased from Qingdao Haiyang chemical Co. Ltd. All commercial reagents and solvents were used without further purification unless otherwise noted. The purity of all compounds screened in biological assays was >95% pure as judged by HPLC. HPLC analysis was obtained on an Hitachi L-2400 system, using a AichromBond-AQ C_{18} column (150 mm × 4.6 mm, 5 μm) with a 1.5 mL/min flow rate using acetonitrile and water solution (v/v=70:30) as the eluent over 30 min.

4.1.1. General procedure for the synthesis of 5a-5r

Preparation of 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone (1)

To a solution of 1-(2,4,6-trihydroxyphenyl)ethanone (11.16 g, 0.06 mol) in dry acetone (150 mL), potassium carbonate (24.84g, 0.18 mol) was added and stirred for 30 min at room temperature. Then dimethyl sulphate (12 mL, 0.12 mol) was added drop-wise, the reaction solution was stirred for 4-5 h at room temperature and monitored by thin-layer chromatograph (TLC). Poured the reaction solution to ice-water(1000 mL) and the precipitate was filtered, washed with water, dried to afford **1** (11.0 g) as white solid in 93.5% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.79 (s, 1H, OH), 6.11 (d, 1H, *J*=2.3 Hz), 6.08 (d, 1H, *J*=2.3 Hz), 3.86 (s, 1H), 3.81 (s, 1H), 2.55 (s, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 203.2, 166.7, 166.4, 163.2, 105.9, 94.1, 91.2, 56.5, 56.1, 33.1.

Preparation of ethyl 4,6-dimethoxy-3-methylbenzofuran-2-carboxylate (2)

To a solution of **1** (3.92 g, 0.02 mol) in dry DMF (50 mL), potassium carbonate (8.28 g, 0.06 mol) was added, the reaction solution was warmed up to 70℃ and stirred for 30 min, then ethyl 2-bromoacetate (3.3 mL, 0.03 mol) was added drop-wise and the reaction solution was stirred for another 2 h at 90°C. Poured the reaction solution to ice-water (500 mL) and the precipitate was filtered, washed with water, dried to afford **2** as brown solid, the resulting residue was then purified by column chromatography on silica to give **2** (1.8 g) as white solid in 34.0% yield. LC-MS m/z: 265.1 $[M+H]^+$, 287.1 $[M+Na]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.82 (d, 1H, *J*= 1.84 Hz), 6.42 (d, 1H, *J*= 1.84 Hz), 4.30 (q, 2H, *J*= 7.1 Hz), 3.87 (s, 3H), 3.82 (s, 3H), 2.61 (s, 3H), 1.32 (t, 3H, *J*=7.1 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 162.2, 159.9, 156.5, 138.7, 126.6, 112.2, 95.4, 88.6, 60.8, 56.2, 14.6, 11.4.

Preparation of ethyl 7-carbamoyl-4,6-dimethoxy-3-methylbenzofuran-2-carboxylate (3)

To a solution of **2** (2.64 g, 0.01 mol) in dry acetonitrile, CSI (2.21 g, 0.015 mol) was added drop-wise under nitrogen atmosphere at 0°C, the solution was warmed up to room temperature and stirred for 3 h. Then 20 mL 2N HCl was added to the reaction solution and stirred for additional 3 h. The precipitate was filtered, washed with water, dried to afford **3** as white solid in 94.0% yield. LC-MS m/z: 308.2 $[M+H]^+$, 330.1 $[M+Na]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.63 (s, 1H), 7.47 (s, 1H), 6.61 (s, 1H), 4.31 (q, 2H, *J*= 7.2 Hz), 3.97 (s, 3H), 3.91(s, 3H), 2.62(s, 3H), 1.32(t, 3H, *J*= 7.2 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.7, 159.8, 158.5, 156.9, 153.3, 139.2, 126.3, 112.2, 104.8, 92.0, 60.9, 57.2, 56.6, 14.7, 11.5

Preparation of 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide (FY0413)

To a solution of **3** (0.31 g, 1 mmol) in acetic acid, 30%HBr was added, the solution was stirred for 5-7 h at 110°C and monitored by thin-layer chromatograph (TLC). Sodium bicarbonate solution was

added to the solution to adjust pH to 7, extracted with ethyl acetate for 3 times, the organic phase was washed with water and brine, dried over Na_2SO_4, concentrated and purified by column chromatography on silica (petroleum ether: ethyl acetate = 3:1) to give **FY0413** as white solid in 39.0% yield. M.p.: 208.2-210.1°C. LC-MS m/z: 222.1 $[M+H]^+$, 244.1 $[M+Na]^+$. IR (KBr, cm^{-1} ν): 3455, 3323, 3195, 2924, 2852, 1633, 1519, 1443, 1328, 1292, 903. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.76 (s, 1H, 6-OH), 8.17 (s, 1H, 7-$CONH_2$), 7.60 (d, 1H, *J*= 7.6 Hz, 2-H), 7.53 (s, 1H, 7-$CONH_2$), 6.34 (s, 1H, 5-H), 3.89 (s, 3H, 4-OCH_3), 2.24 (d, 3H, 3-CH_3, *J*= 2.2 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.1, 163.2, 158.4, 153.5, 139.4, 115.5, 109.9, 94.7, 93.5, 56.0, 9.4.

Preparation of 7-carbamoyl-4,6-dimethoxy-3-methylbenzofuran-2-carboxylic acid (4)

Intermediate **3** (4.61 g, 0.015 mol) was placed on 50 mL mixed solution of methanol and NaOH (1.8 g/ 5 mL H_2O), the solution was stirred for 2 h at 60°C, then poured the solution into 500 mL cold water and adjusted the pH to 2 with 2N HCl, filtrated and washed the cake with water, dried to afford **4** as white solid in 90.0% yield. LC-MS m/z: 277.9 $[M-H]^-$.

Preparation of ethyl 7-carbamoyl-6-hydroxy-4-methoxy-3-methylbenzofuran-2-carboxylate (5a)

To a solution of **3** (1.0 g, 3.26 mmol) in dry DCM, BBr_3 (0.6 mL, 6.5 mmol) was added drop-wise under nitrogen atmosphere at -40°C and stirred for 4 h, then warmed up to room temperature and stirred for additional 2 h. The reaction was quenched with water and the DCM was removed by evaporators, then sodium bicarbonate solution was added to the solution to adjust pH to 7. The precipitate was filtered and recrystallized with ethyl alcohol to afford **5a (0.45 g)** as white solid in 47.0% yield. M.p.: 198.0-199.6°C. IR (KBr, cm^{-1} ν): 3457, 3330, 3210, 2923, 2851, 1721, 1633, 1520, 1435, 1327,1280, 926. LC-MS: 294.2 $[M+H]^+$, 316.1 $[M+Na]^+$, 332.0 $[M+K]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.98 (s, 1H, 6-OH), 8.42 (s, 1H, 7-$CONH_2$), 7.16 (s, 1H, 7-$CONH_2$), 6.39 (m, 1H, 5-H), 4.33 (q, 2H, *J*= 4.3 Hz, -$COOCH_2CH_3$), 3.92 (d, 3H, *J*= 3.9 Hz), 2.56 (s, 3H, 3-CH_3), 1.33 (t, 3H, *J*= 1.3 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 169.7, 166.0, 159.6, 158.9, 152.8, 138.3, 126.3, 110.4, 95.8, 93.0, 60.6, 56.3, 14.1, 10.7.

General procedure for the synthesis of 5b-5r

To a solution of 7-carbamoyl-6-hydroxy-4-methoxy-3-methylbenzofuran-2-carboxylic acid (**4**) (0.279 g, 1 mmol) in DMSO, HATU (0.52 g, 1.3 mmol) and DIEA (0.3mL, 1.9 mmol) were added at 25°C, then corresponding amino side chain (1 mmol) was added. After thin-layer chromatograph (TLC) indicating the reaction completed, poured the solution to 150 mL ice-water and stirred for 30 min, the

precipitate was filtered and dried. Then these residues were dissolved in dry DCM under nitrogen atmosphere, BBr_3 (2 eq.) was added drop-wise under nitrogen atmosphere at -40°C and stirred for 4 h, then warmed up to room temperature and stirred for additional 2 h. The reaction was quenched with water and the DCM was removed by evaporators, then sodium bicarbonate solution was added to the solution to adjust pH to 7. The precipitate was filtered and recrystallized with ethyl alcohol to afford **5b-5r**.

Preparation of 6-hydroxy-4-methoxy-N^2,3-dimethylbenzofuran-2,7-dicarboxamide (5b)

Yield 58.0%. M.p.: 219.0-220.7°C. IR (KBr, cm^{-1} ν): 3431, 2923, 2852, 1622, 1536, 1451, 1329, 1290, 872. LC-MS m/z: 279.2 $[M+H]^+$, 301.1 $[M+Na]^+$, 276.9 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.50 (s, 1H, 6-OH), 8.71 (t, 1H, *J*= 8.7 Hz, 2-CONH-), 8.31 (s, 1H, 7-$CONH_2$), 7.91 (s, 1H, 7-$CONH_2$), 6.39 (s, 1H, 5-H), 3.92 (s, 1H, 4-OCH_3), 2.80 (d, 3H, *J*= 2.79 Hz, $NHCH_3$), 2.61 (s, 3H, 3-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 169.8, 165.7, 159.3, 151.7, 141.5, 122.0, 110.8, 95.7, 93.1, 56.1, 25.3, 10.6.

Preparation of 6-hydroxy-4-methoxy-N^2,N^2,3-trimethylbenzofuran-2,7-dicarboxamide (5c)

Yield 53.0%. M.p.: 227.3-229.9°C. IR (KBr, cm^{-1} ν): 3476, 3183, 2971, 2923, 1625, 1526, 1450, 1324, 1209, 821. LC-MS m/z: 293.2 $[M+H]^+$, 315.1 $[M+Na]^+$, 291.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.93 (s, 1H, 6-OH), 8.26 (s, 1H, 7-$CONH_2$), 7.40 (s, 1H, 7-$CONH_2$), 6.41 (s, 1H, 5-H), 3.92 (s, 3H, 4-OCH_3), 3.05 (brs, 6H, -NMe_2), 2.39 (s, 3H, 3-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 169.9, 164.6, 160.4, 159.0, 151.9, 142.1, 119.6, 110.1, 95.4, 93.3, 56.2, 10.38.

Preparation of N^2-(2-(dimethylamino)ethyl)-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (5d)

Yield 64.0%. M.p.: 256.1-258.6°C. IR (KBr, cm^{-1} ν): 3434, 3340, 2922, 2852, 1621, 1511, 1455, 1329, 1208, 820. LC-MS m/z: 336.2 $[M+H]^+$, 358.2 $[M+Na]^+$, 334.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.50 (s, 1H, 6-OH), 8.75 (t, 1H, *J*= 8.7 Hz, 2-CONH-), 8.32 (s, 1H, 7-$CONH_2$), 7.91 (s, 1H, 7-$CONH_2$), 6.40 (s, 1H, 5-H), 3.92 (s, 3H, 4-OCH_3), 3.36 (m, 4H), 2.61 (s, 3H, 3-CH_3), 2.44 (t, 2H, *J*= 2.4 Hz), 2.22 (s, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 166.3, 159.8, 159.3, 152.2, 141.8, 122.8, 111.4, 96.2, 93.6, 58.8, 56.7, 45.7, 36.9, 11.1.

Preparation of N^2-cyclopentyl-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (5e)

Yield 42.0%. M.p.: 229.5-230.8°C. IR (KBr, cm^{-1} ν): 3475, 2921, 2852, 1623, 1526, 1450, 1328, 1208, 821. LC-MS m/z: 333.2 $[M+H]^+$, 355.2 $[M+Na]^+$, 371.2 $[M+K]^+$, 331.0 $[M-H]^-$. ^{1}H-NMR (400

MHz, DMSO-d_6) δ: 14.41 (s, 1H, 6-OH), 8.53 (d, 1H, *J*=8.5 Hz, 2-CONH-), 8.28 (s, 1H, 7-$CONH_2$), 8.00 (s, 1H, 7-$CONH_2$), 6.39 (s, 1H, 5-H), 4.22 (m, 1H), 3.91 (s, 3H, 4-OCH_3), 2.60 (s, 1H, 3-CH_3), 1.93 (m, 2H), 1.71 (m, 2H), 1.55 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 169.8, 165.6, 159.3, 158.7, 151.7, 141.6, 122.2, 111.0, 95.7, 93.3, 56.2, 50.2, 31.9, 23.7, 10.7.

Preparation of N^2-cyclohexyl-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (5f)

Yield 51.0%. M.p.: 202.2-204.1°C. IR (KBr, cm^{-1} ν): 3435, 3379, 2923, 2852, 1620, 1525, 1450, 1317, 1283, 822, LC-MS m/z: 347.3 $[M+H]^+$, 369.3 $[M+Na]^+$, 345.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.39 (s, 1H, 6-OH), 8.45 (d, 1H, *J*=8.5 Hz, 2-CONH-), 8.24 (s, 1H, 7-$CONH_2$), 7.97 (s, 1H, 7-$CONH_2$), 6.37 (s, 1H, 5-H), 3.89 (s, 3H, 4-OCH_3), 3.75 (m, 1H), 2.58 (s, 3H, 3-CH_3), 1.81 (d, 2H, *J*= 1.8 Hz), 1.74 (d, 2H, *J*= 1.7 Hz), 1.33 (m, 6H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-2-(morpholine-4-carbonyl)benzofuran-7-carboxamide (5g)

Yield 44.0%. M.p.: 171.3-172.7°C. IR (KBr, cm^{-1} ν): 3460, 3326, 3205, 2920, 2851, 1632, 1517, 1431, 1329, 1281, 826. LC-MS m/z: 335.5 $[M+H]^+$, 357.5 $[M+Na]^+$, 333.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.90 (s, 1H, 6-OH), 8.27 (s, 1H, 7-$CONH_2$), 7.38 (s, 1H, 7-$CONH_2$), 6.42 (s, 1H, 5-H), 3.92 (s, 3H, 4-OCH_3), 3.64 (m, 4H), 3.60 (m, 4H), 2.39 (s, 3H, 3-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 165.1, 159.8, 159.5, 152.5, 142.0, 120.6, 110.6, 96.0, 93.9, 66.6, 56.7, 10.8.

Preparation of 6-hydroxy-4-methoxy-3-methyl-2-(piperidine-1-carbonyl)benzofuran-7-carboxamide (5h)

Yield 43.0%. M.p.: 190.4-192.3°C. IR (KBr, cm^{-1} ν): 3477, 3328, 2924, 2852, 1613, 1516, 1445, 1327, 1269, 822. LC-MS m/z: 333.2 $[M+H]^+$, 355.2 $[M+Na]^+$, 371.2 $[M+K]^+$, 331.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.87 (s, 1H, 6-OH), 8.30 (s, 1H, 7-$CONH_2$), 7.33 (s, 1H, 7-$CONH_2$), 6.41 (s, 1H), 3.92 (s, 3H), 3.52 (br, 4H), 2.36 (s, 3H), 1.57 (m, 4H), 1.24 (br, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 169.9, 164.5, 159.1, 159.0, 151.9, 142.1, 119.1, 110.1, 95.4, 93.4, 56.2, 24.1, 10.3.

Preparation of 2-(4-ethylpiperazine-1-carbonyl)-6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide (5i)

Yield 83.0%. M.p.: 204.1-205.6°C. IR (KBr, cm^{-1} ν): 3421, 3204, 2922, 2851, 1622, 1515, 1457, 1326, 1207, 824. LC-MS m/z: 362.2 $[M+H]^+$, 384.2 $[M+Na]^+$, 400 $[M+K]^+$, 360.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.88 (s, 1H, 6-OH), 8.28 (s, 1H, 7-$CONH_2$), 7.33 (s, 1H, 7-$CONH_2$), 6.41 (s, 1H), 3.92 (s, 3H), 3.57 (br, 4H), 2.42 (br, 4H), 2.37 (s, 3H), 1.23 (s, 2H), 1.02 (t, 3H, *J*= 1.0 Hz).

Preparation of 6-hydroxy-4-methoxy-3-methyl-2-(4-phenylpiperazine-1-carbonyl)benzofuran-7-carboxamide (5j)

Yield 68.0%. M.p.: 246.4-248.2°C. IR (KBr, cm^{-1} ν): 3470, 3337, 3186, 2923, 2852, 1634, 1515, 1463, 1387, 1211, 823. LC-MS m/z: 410.3 $[M+H]^+$, 432.2 $[M+Na]^+$, 448.2 $[M+K]^+$, 408.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.90 (s, 1H, 6-OH), 8.27 (s, 1H, 7-$CONH_2$), 7.44 (s, 1H, 7-$CONH_2$), 7.24 (t, 2H, *J*= 7.2 Hz), 6.97 (d, 2H, *J*= 7.0 Hz), 6.82 (t, 1H, *J*= 6.8 Hz), 6.41 (s, 1H), 3.92 (s, 3H), 3.73 (br, 4H), 3.22 (br, 4H), 2.41 (s, 3H).

Preparation of 2-(4-(4-fluorophenyl)piperazine-1-carbonyl)-6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide (5k)

Yield 32.0 %. M.p.:249.4-250.9°C. IR (KBr, cm^{-1} ν): 3477, 2921, 2852, 1628, 1509, 1460, 1326, 1209, 752. LC-MS m/z: 450.2 $[M+Na]^+$, 426.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.90 (s, 1H, 6-OH), 8.28 (br, 1H, 7-$CONH_2$), 7.41 (br, 1H, 7-$CONH_2$), 7.08 (m, 2H), 7.00 (m, 2H), 6.43 (s, 1H), 3.93 (s, 3H), 3.73 (br, 4H), 3.17 (br, 4H), 2.41 (s, 3H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-2-(4-(3-(trifluoromethyl)phenyl)piperazine-1-carbonyl)benzofuran-7-carboxamide (5l)

Yield 61.0 %. M.p.: 228.6-230.1°C. IR (KBr, cm^{-1} ν): 3475, 3329, 3186, 2922, 2864, 1631, 1496, 1443, 1324, 1211, 884, 790. LC-MS m/z: 474.3 $[M+H]^+$, 496.3 $[M+Na]^+$, 512.2 $[M+K]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.91 (s, 1H, 6-OH), 8.28 (br, 1H, 7-$CONH_2$), 7.45 (t, 1H, *J*= 8.0 Hz), 7.42 (br, 1H, 7-$CONH_2$), 7.25 (d, 1H, *J*= 8.6 Hz), 7.22 (s, 1H), 7.11 (d, 1H, *J*= 7.2 Hz), 6.43 (s, 1H), 3.93 (s, 3H), 3.74 (br, 4H), 3.35 (br, 4H), 2.42 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 165.2, 159.8, 159.6, 152.5, 152.4, 142.1, 130.5, 126.2, 123.5, 119.5, 115.5, 111.9, 110.7, 96.0, 93.9, 56.7, 48.3, 48.2, 10.9.

Preparation of 6-hydroxy-4-methoxy-3-methyl-N^2-phenylbenzofuran-2,7-dicarboxamide (5m)

Yield 46.0%. M.p.: 286.8-287.7°C. IR (KBr, cm^{-1} ν): 3431, 3357, 3200, 2922, 2852, 1622, 1521, 1443, 1316, 1207, 754, 723. LC-MS m/z: 341.2 $[M+H]^+$, 363.2 $[M+Na]^+$, 339.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.50 (s, 1H, 6-OH), 10.35 (s, 1H), 8.32 (s, 1H, 7-$CONH_2$), 8.13 (s, 1H, 7-$CONH_2$), 7.66 (d, 2H, *J*= 8.0 Hz), 7.37 (t, 2H, *J*= 8.0 Hz), 7.14 (t, 1H, *J*=8.0 Hz), 6.41 (s, 1H), 3.92 (s, 3H), 2.65 (s, 3H).

Preparation of N^2-(4-fluoro-3-(trifluoromethyl)phenyl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (5n)

Yield 49.0%. M.p.: 271.6-272.5°C. IR (KBr, cm^{-1} ν): 3430, 2921, 2852, 1622, 1531, 1452, 1322, 1210, 830, 619. LC-MS m/z: 443.2 $[M+H]^+$, 465.2 $[M+Na]^+$, 441.1 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.56 (s, 1H, 6-OH), 10.63 (s, 1H), 8.37 (s, 1H, 7-$CONH_2$), 8.26 (d, 1H, *J*= 4.0 Hz), 8.07 (s, 1H, 7-$CONH_2$), 8.02 (dd, 1H, *J*= 2.2 Hz, *J*= 8.8 Hz), 7.70 (d, 1H, *J*= 8.7 Hz), 6.40 (s, 1H), 3.91 (s, 3H), 2.64 (s, 3H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-*N*^{2}-(4-methyl-3-(trifluoromethyl)phenyl) benzofuran-2,7-dicarboxamide (5o)

Yield 57.0%. M.p.: 294.3-296.1°C. IR (KBr, cm^{-1} ν): 3457, 3358, 2922, 2852, 1623, 1505, 1451, 1320, 1211, 824, 699. LC-MS m/z: 445.2 $[M+Na]^+$, 421.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.57 (s, 1H, 6-OH), 10.53 (s, 1H), 8.38 (s, 1H, 7-$CONH_2$), 8.12 (s, 1H, 7-$CONH_2$), 8.07 (d, 1H, *J*= 4.0 Hz), 7.89 (dd, 1H, *J*= 2.2 Hz, *J*= 8.8 Hz), 7.45 (d, 1H, *J*= 8.7 Hz), 6.44 (s, 1H), 3.94 (s, 3H), 2.67 (s, 3H), 2.43 (s, 3H).

Preparation of *N*^{2}-benzyl-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (5p)

Yield 52.0%. M.p.: 294.0-295.7°C. IR (KBr, cm^{-1} ν): 3427, 3332, 2923, 2852, 1622, 1534, 1450, 1329, 1212, 822, 748. LC-MS m/z: 355.2 $[M+H]^+$, 377.2 $[M+Na]^+$, 353.2 $[M-H]^-$.

Preparation of *N*^{2}-benzyl-6-hydroxy-4-methoxy-*N*^{2},3-dimethylbenzofuran-2,7-dicarboxamide (5q)

Yield 34.0%. M.p.: 231.3-232.8°C. IR (KBr, cm^{-1} ν): 3471, 3321, 3164, 2925, 2852, 1636, 1516, 1446, 1324, 1210, 735, 712. LC-MS m/z: 369.3 $[M+H]^+$, 391.3 $[M+Na]^+$, 367.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.90 (s, 1H), 8.20 (br, 1H), 7.995 (br, 1H), 7.37 (m, 3H), 7.30 (m, 2H), 6.39 (s, 1H), 4.64 (s, 2H), 3.90 (s, 3H), 2.98 (br, 3H), 2.41 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 165.2, 159.5, 152.4, 142.5, 137.5, 129.1, 127.9, 120.9, 110.7, 96.0, 93.9, 56.7, 10.9.

Preparation of *N*^{2}-(3-fluorobenzyl)-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (5r)

Yield 28.0%. M.p.: 266.3-267.2°C. IR (KBr, cm^{-1} ν): 3430, 3291, 2922, 2851, 1621, 1533, 1452, 1327, 1212, 821, 786. LC-MS m/z: 373.2 $[M+H]^+$, 395.2 $[M+Na]^+$, 371.1 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.51 (s, 1H, 6-OH), 9.33 (t, 1H, *J*= 6.0 Hz), 8.28 (s, 1H, 7-$CONH_2$), 7.91 (s, 1H, 7-$CONH_2$), 7.37 (q, 1H, *J*= 7.9 Hz), 7.14 (m, 2H), 7.07 (m, 1H), 6.39 (s, 1H), 4.49 (d, 2H, *J*= 6.0 Hz), 3.90 (s, 3H), 2.61 (s, 3H).

Preparation of tert-butyl 4-(7-carbamoyl-4,6-dimethoxy-3-methylbenzofuran-2-carboxamido) piperidine-1-carboxylate (6a)

To a solution of **4** (2.79 g, 10 mmol) in DMSO, HATU (5.2 g, 13 mmol) and DIEA (3 mL, 19 mmol) were added at 25°C, then 1-Boc-4-aminopiperidine (2.0 g, 10 mmol) was added. After thin-layer chromatograph (TLC) indicating the reaction completed, poured the solution to 150 mL ice-water and stirred for 30 min, the precipitate was filtered and dried to afford **6a** as white solid in 65.0% yield. LC-MS m/z: 460.1 $[M-H]^-$.

Preparation of tert-butyl 4-((7-carbamoyl-4,6-dimethoxy-3-methylbenzofuran-2-carboxamido) methyl)piperidine-1-carboxylate (6b)

To a solution of **4** (2.79 g, 10 mmol) in DMSO, HATU (5.2 g, 13 mmol) and DIEA (3 mL, 19 mmol) were added at 25°C, then 1-Boc-4-(aminomethyl)piperidine (2.14 g, 10 mmpl) was added. After thin-layer chromatograph (TLC) indicating the reaction completed, poured the solution to 150 mL ice-water and stirred for 30 min, the precipitate was filtered and dried to afford **6a** as white solid in 58.0% yield. LC-MS m/z: 474.2 $[M-H]^-$.

Preparation of 4,6-dimethoxy-3-methyl-N^2-(piperidin-4-yl)benzofuran-2,7-dicarboxamide (7a)

To a solution of **6a** (5 mmol) in dry DCM, saturated HCl/ ethyl acetate solution (10 mL) was added and stirred for 1 h at room temperature. The precipitate was filtered and dried to afford **7a** as white solid in 92.0% yield. LC-MS m/z: 362.3 $[M+H]^+$, 384.2 $[M+Na]^+$.

Preparation of 4,6-dimethoxy-3-methyl-N^2-(piperidin-4-ylmethyl)benzofuran-2,7-dicarboxamide (7b)

To a solution of **6b** (5 mmol) in dry DCM, saturated HCl/ ethyl acetate solution (10 mL) was added and stirred for 1 h at room temperature. The precipitate was filtered and dried to afford **7b** as white solid in 88.0% yield. LC-MS m/z: 376.3 $[M+H]^+$, 398.2 $[M+Na]^+$.

General procedure for the synthesis of 8a-8p

To a solution of **7a/7b** (1 mmol) in DMF, K_2CO_3 (0.828 g, 6 mmol) was added and the solution was stirred for 0.5 h at 80°C, then corresponding substituted benzyl chloride (benzyl bromide) or substituted benzoyl chloride was added and stirred for additional 5-6 h. After thin-layer chromatograph (TLC) indicating the reaction completed, poured the solution to 150 mL ice-water and stirred for 30 min, the precipitate was filtered as yellowish white solid, washed the precipitate with diethyl ether. Dissolving these solid(0.5 mmol) in dry DCM under nitrogen atmosphere, BBr_3 (1 mmol) was added drop-wise under nitrogen atmosphere at -40°C and stirred for 4 h, then warmed up to room temperature and stirred for additional 2 h. The reaction was quenched with water and the DCM was removed by

evaporators, then sodium bicarbonate solution was added to the solution to adjust pH to 7. The precipitate was filtered and recrystallized with ethyl alcohol, purified by column chromatography on silica (DCM: methyl alcohol = 20:1) to give compounds **8a-8p** as off-white solid.

Preparation of 6-hydroxy-4-methoxy-3-methyl-N^2-(1-(pyridin-4-ylmethyl)piperidin-4-yl) benzofuran-2,7-dicarboxamide (8a)

Yield 11.0%. M.p.: 283.4-284.7°C. IR (KBr, cm^{-1} ν): 3428, 3270, 3185, 2923, 2852, 1661, 1508, 1451, 1347, 1289. LC-MS m/z: 439.3 $[M+H]^+$, 461.3 $[M+Na]^+$, 437.1 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 14.44 (s, 1H, 6-OH), 8.51 (d, 2H, J= 5.3 Hz), 8.48 (d, 1H, J= 8.0 Hz), 8.29 (s, 1H), 7.97 (s, 1H), 7.33 (d, J= 5.2 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.80 (m, 1H), 3.53 (s, 2H), 2.83 (d, 2H, J= 10.3 Hz), 2.60 (s, 3H, 3-Me), 2.08 (t, 2H, J= 11.1 Hz), 1.80 (d, 2H, J= 9.8 Hz), 1.68 (q, 2H, J= 11.8 Hz).

Preparation of N^2-(1-((6-chloropyrimidin-4-yl)methyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methylbenzofuran-2,7-dicarboxamide (8b)

Yield 23.0%. M.p.: 310.2-311.6°C. IR (KBr, cm^{-1} ν): 3464, 3323, 3191, 2921, 2852, 1652, 1520, 1493, 1340, 1300，LC-MS m/z: 460.1 $[M+H]^+$, 482.1 $[M+Na]^+$, 457.9 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 14.42 (s, 1H, 6-OH), 8.42 (d, 1H, J= 8.0 Hz), 8.36 (s, 1H), 8.27 (s, 1H), 7.88 (s, 1H), 7.05 (s, 1H), 6.39 (s, 1H), 4.16 (m, 1H), 3.91 (s, 3H, 4-OMe), 3.08 (t, 2H, *J*= 12.6 Hz), 2.61 (s, 3H, 3-Me), 1.89 (d, 2H, *J*= 9.8 Hz), 1.52 (m, 2H), 1.23 (m, 2H).

Preparation of N^2-(1-(2-fluorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl-benzofuran-2,7-dicarboxamide (8c)

Yield 43.0%. M.p.: 251.2-252.9°C. IR (KBr, cm^{-1} ν): 3441, 2922, 2851, 1656, 1524, 1490, 1322, 1291, 749.1. LC-MS m/z: 456.2 $[M+H]^+$, 478.2 $[M+Na]^+$,454.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 14.43 (s, 1H, 6-OH), 8.45 (d, 1H, J= 7.4 Hz), 8.27 (s, 1H), 7.95 (s, 1H), 7.41 (m, 1H), 7.32 (m, 1H), 7.18 (m, 2H), 6.39 (s, 1H), 3.91 (s, 3H, 4-OMe), 3.79 (br, 1H), 3.54 (s, 2H), 2.86 (d, 2H, *J*= 9.1 Hz), 2.60 (s, 3H, 3-Me), 2.07 (t, 2H, *J*= 9.6 Hz), 1.79 (d, 2H, *J*= 10.2 Hz), 1.63 (m, 2H).

Preparation of N^2-(1-(4-fluorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl-benzofuran-2,7-dicarboxamide (8d)

Yield 30.0%. LC-MS m/z: 456.2 $[M+H]^+$, 478.2 $[M+Na]^+$, 454.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.43 (s, 1H, 6-OH), 8.46 (d, 1H, *J*= 6.5 Hz), 8.28 (s, 1H), 7.96 (s, 1H), 7.34 (br, 2H),

7.15 (m, 2H), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.80 (br, 1H), 3.46 (s, 2H), 2.82 (br, 2H), 2.60 (s, 3H, 3-Me), 2.02 (m, 2H), 1.78 (m, 2H), 1.65 (m, 2H).

Preparation of N^2-((1-(4-fluorobenzyl)piperidin-4-yl)methyl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8e)

Yield 21.0%. M.p.: 189.1-191.0°C. IR (KBr, cm^{-1} ν): 3432, 2921, 2851, 1621, 1512, 1451, 1329, 1286, 823. LC-MS m/z: 470.7 $[M+H]^+$, 468.4 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.51 (s, 1H, 6-OH), 8.82 (t, 1H, *J*= 4.5 Hz), 8.30 (s, 1H), 7.90 (s, 1H), 7.56 (br, 2H), 7.30 (br, 2H), 6.40 (s, 1H), 4.08 (q, 1H, *J*= 5.0 Hz), 3.91 (s, 3H, 4-OMe), 3.37 (br, 2H), 3.17 (m, 4H), 2.92 (br, 2H), 2.61 (s, 3H, 3-Me), 1.83 (br, 2H), 1.44 (br, 2H).

Preparation of N^2-((1-(4-fluorobenzoyl)piperidin-4-yl)methyl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8f)

Yield 27.0%. M.p.: 173.6-174.4°C. IR (KBr, cm^{-1} ν): 3434, 2922, 2852, 1621, 1512, 1450, 1330, 1284, 878.3, 846.5, 823.6. LC-MS m/z: 484.3 $[M+H]^+$, 506.2 $[M+Na]^+$, 482.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 14.49 (s, 1H, 6-OH), 8.81 (t, 1H, J= 5.5 Hz), 8.29 (s, 1H), 7.92 (s, 1H), 7.44 (t, 2H, *J*= 8.2 Hz), 7.27 (t, 2H, *J*= 8.7 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.19 (s, 2H), 3.01 (m, 1H), 2.61 (s, 3H), 1.60-1.86 (m, 4H), 1.18 (m, 4H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-N^2-(1-(4-methylbenzyl)piperidin-4-yl)benzofuran -2,7-dicarboxamide (8g)

Yield 26.0%. M.p.: 242.7-243.7°C. IR (KBr, cm^{-1} ν): 3425, 2921, 2851, 1623, 1515, 1451, 1328, 1263，822.9. LC-MS m/z: 452.3 $[M+H]^+$, 474.3 $[M+Na]^+$, 450.1 $[M-H]^-$.

Preparation of N^2-(1-(4-chlorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl-benzofuran-2,7-dicarboxamide (8h)

Yield 32.0%. M.p.: 245.4-246.9°C. IR (KBr, cm^{-1} ν): 3474, 2922, 2851, 1657, 1523, 1491, 1323, 1290, 822. LC-MS m/z: 472.2 $[M+H]^+$, 494.2 $[M+Na]^+$, 470.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d6) δ: 14.43 (s, 1H, 6-OH), 8.45 (d, 1H, J= 7.9 Hz), 8.28 (s, 1H), 7.96 (s, 1H), 7.38 (d, 2H, J= 7.4 Hz), 7.33 (d, 2H, J= 7.4 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.80 (br, 1H), 3.47 (s, 2H), 2.82 (d, 2H, J= 9.1 Hz), 2.60 (s, 3H, 3-Me), 2.03 (t, 2H, J= 10.2 Hz), 1.77 (m, 2H), 1.63 (m, 2H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-N^2-(1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)benzofuran-2,7-dicarboxamide (8i)

Yield 21.0%. M.p.: 231.5-232.2°C. IR (KBr, cm^{-1} ν): 3422, 2922, 2852, 1625, 1513, 1450, 1327, 1292, 821. LC-MS m/z: 506.4 $[M+H]^+$, 528.3 $[M+Na]^+$, 504.3 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.43 (s, 1H, 6-OH), 8.46 (d, 1H, *J*= 8.0 Hz), 8.28 (s, 1H), 7.96 (s, 1H), 7.69 (d, 2H, *J*= 8.0 Hz), 7.54 (d, 2H, *J*= 8.0 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.81 (br, 1H), 3.58 (s, 1H), 2.83 (d, 2H, *J*= 11.2 Hz), 2.60 (s, 3H, 3-Me), 2.07 (t, 2H, *J*= 11.3 Hz), 1.80 (d, 2H, *J*= 11.1 Hz), 1.61-1.70 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 166.2, 159.8, 158.8, 152.2, 141.8, 129.8, 125.6, 122.9, 111.4, 96.2, 93.7, 56.6, 52.8, 46.5, 31.8, 11.1.

Preparation of 6-hydroxy-4-methoxy-3-methyl-*N*^{2}-(1-(4-(trifluoromethyl)benzoyl)piperidin-4-yl)benzofuran-2,7-dicarboxamide (8j)

Yield 34.0%. M.p.: 215.7-216.2°C. IR (KBr, cm^{-1} ν): 3449, 2923, 2852, 1658, 1519, 1452, 1324, 1276, 820.1, 848. LC-MS m/z: 520.1 $[M+H]^+$, 542.2 $[M+Na]^+$, 518.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.15 (s, 1H, 6-OH), 8.52 (d, 1H, *J*= 7.8 Hz), 8.31 (s, 1H), 7.94 (s, 1H), 7.84 (d, 2H, *J*= 8.0 Hz), 7.60 (d, 2H, *J*= 8.0 Hz), 6.40 (s, 1H, 5-H), 4.52 (m, 1H), 4.12 (m, 1H), 3.91 (s, 3H, 4-OMe), 3.53 (m, 1H), 2.94 (m, 1H), 2.61 (s, 3H, 3-Me), 1.95 (m, 1H), 1.81 (m, 1H), 1.50-1.60 (m, 2H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-*N*^{2}-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl)benzofuran-2,7-dicarboxamide (8k)

Yield 28.0%. M.p.: 219.4-220.7°C. IR (KBr, cm^{-1} ν): 3437, 2922, 2851, 1620, 1524, 1452, 1329, 1291, 757. LC-MS m/z: 506.2 $[M+H]^+$, 504.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.45 (s, 1H, 6-OH), 8.46 (d, 1H, *J*= 7.3 Hz), 8.30 (s, 1H), 7.96 (s, 1H), 7.60-7.67 (m, 4H, phenyl H), 6.40 (s, 1H, 5-H), 3.92 (s, 3H, 4-OMe), 3.83 (m, 1H), 3.60 (s, 2H), 2.84 (d, 2H, *J*= 10.0 Hz), 2.61 (s, 3H, 3-Me), 2.08 (t, 2H, *J*= 12.0 Hz), 1.79 (m, 2H), 1.66 (m, 2H).

Preparation of 6-hydroxy-4-methoxy-3-methyl-*N*^{2}-((1-(3-(trifluoromethyl)benzyl)piperidin-4-yl)methyl)benzofuran-2,7-dicarboxamide (8l)

Yield 28.0%. M.p.: 181.5-183.4°C. IR (KBr, cm^{-1} ν): 3426, 2923, 2852, 1621, 1514, 1451, 1329, 1286, 877, 821. LC-MS m/z: 520.6 $[M+H]^+$, 518.4 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.50 (s, 1H, 6-OH), 9.43 (br, 1H, NH), 8.82 (s, 1H, NH_2), 8.30 (s, 1H, NH_2), 7.70-7.81 (m, 4H, phenyl H), 6.40 (s, 1H), 4.40 (br, 1H), 3.91 (s, 3H, 4-OMe), 3.38 (m, 2H), 3.18 (s, 2H), 2.96 (m, 2H), 2.61 (s, 3H, 3-Me), 1.84 (br, 4H), 1.44 (br, 2H).

Preparation of *N*^{2}-(1-(2,6-dichlorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8m)

Yield 28.0%. M.p.: 224.6-225.3°C. IR (KBr, cm^{-1} ν): 3463, 2921, 2851, 2764, 1660, 1526, 1323, 1286, 928. LC-MS m/z: 506.3 $[M+H]^+$, 528.3 $[M+Na]^+$, 504.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.43 (s, 1H, 6-OH), 8.39 (d, 1H, *J*= 8.0 Hz), 8.25 (s, 1H), 7.92 (s, 1H), 7.46 (d, 2H, 8.0 Hz), 7.33 (t, 1H, *J*= 7.6 Hz), 6.39 (s, 1H, 5-OH), 3.91 (s, 3H, 4-OMe), 3.82 (m, 1H), 3.70 (s, 2H), 2.83 (d, 2H, *J*= 11.3 Hz), 2.60 (s, 3H, 3-Me), 2.26 (t, 2H, *J*= 10.5 Hz), 1.76 (d, 2H, *J*= 10.0 Hz), 1.55-1.63 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.6, 166.2, 159.7, 158.7, 152.2, 141.8, 136.6, 134.5, 130.2, 129.0, 122.9, 111.4, 96.2, 93.7, 56.6, 56.4, 52.7, 46.3, 31.9, 11.1.

Preparation of N^2-(1-(2,4-dichlorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8n)

Yield 40.0%. M.p.: 236.8-237.4°C. IR (KBr, cm^{-1} ν): 3472, 2922, 2852, 1625, 1525, 1448, 1325, 1292, 822. LC-MS m/z: 506.3 $[M+H]^+$, 528.3 $[M+Na]^+$, 504.2 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.43 (s, 1H, 6-OH), 8.44 (d, 1H, *J*= 8.0 Hz), 8.28 (s, 1H), 7.95 (s, 1H), 7.59 (d, 1H, *J*= 1.4 Hz), 7.50 (d, 1H, *J*= 8.4 Hz), 7.43 (dd, 1H, *J*= 1.4 Hz, 8.4 Hz), 6.39 (s,1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.83 (m, 1H), 3.56 (s, 2H), 2.84 (d, 2H, *J*= 10.7 Hz), 2.60 (s, 3H, 3-Me), 2.15 (t, 2H, *J*= 10.9 Hz), 1.79 (d, 2H, *J*= 9.9 Hz), 1.64-1.69 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 166.2, 159.8, 158.8, 152.2, 141.9, 134.6, 132.3, 129.1, 127.7, 122.9, 111.4, 96.2, 93.7, 58.5, 56.6, 52.8, 46.5, 31.9, 11.1.

Preparation of N^2-(1-(2,3-dichlorobenzyl)piperidin-4-yl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8o)

Yield 34.0%. M.p.: 159.6-160.4°C. IR (KBr, cm^{-1} ν): 3471, 2923, 2852, 1655, 1523, 1450, 1325, 1292, 823.1. LC-MS m/z: 506.3 $[M+H]^+$, 528.3 $[M+Na]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.43 (s, 1H, 6-OH), 8.46 (d, 1H, *J*= 8.0 Hz), 8.29 (s, 1H), 7.96 (s, 1H), 7.56 (d, 1H, *J*= 7.4 Hz), 7.49 (d, 1H, *J*= 7.4 Hz), 7.38 (t, 1H, *J*= 7.8 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.84 (m, 1H), 3.66 (s, 2H), 2.90 (d, 2H, *J*= 9.8 Hz), 2.60 (s, 3H, 3-Me), 2.23 (m, 2H), 1.82 (d, 2H, *J*= 10.1 Hz), 1.68 (q, 2H, *J*= 10.7 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.3, 166.2, 159.8, 158.8, 152.2, 141.9, 132.3, 129.6, 128.4, 122.9, 111.4, 96.2, 93.7, 59.6, 56.7, 52.8, 46.4, 31.7, 11.1.

Preparation of N^2-((1-(3,4-dichlorobenzyl)piperidin-4-yl)methyl)-6-hydroxy-4-methoxy-3-methyl benzofuran-2,7-dicarboxamide (8p)

Yield 41.0%. M.p.: 244.6-245.5°C. IR (KBr, cm^{-1} ν): 3417, 3271, 2922, 2851, 1656, 1530, 1452, 1339, 1288, 823. LC-MS m/z: 520.6 $[M+H]^+$, 518.3 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.49 (s, 1H, 6-OH), 8.77 (t, 1H, *J*= 5.5 Hz), 8.27 (s, 1H), 7.92 (s, 1H), 7.56 (t, 2H, *J*= 10.4 Hz), 7.29 (d, 1H,

J= 7.8 Hz), 6.39 (s, 1H, 5-H), 3.91 (s, 3H, 4-OMe), 3.45 (s, 2H), 3.16 (t, 2H, *J*= 5.7 Hz), 2.78 (d, 2H, *J*= 8.8 Hz), 2.60 (s, 3H, 3-Me), 1.93 (br, 2H), 1.65 (d, 2H, *J*= 10.9 Hz), 1.56 (m, 1H), 1.23 (m, 2H).

Preparation of 4,6-dimethoxy-3-methyl-*N*^{2}-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl) benzofuran-2,7-dicarboxamide (17a)

17a was synthesized by the method shown in **Scheme 2** as white solid. Yield 51.0%. M.p.: 185.1-186.5°C, LC-MS: 520.3 $[M+H]^+$, 542.3 $[M+Na]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.75 (d, 1H, *J*= 8.4 Hz), 7.66 (brs, 1H), 7.56-7.63 (m, 4H), 7.38 (brs, 1H), 6.59 (s, 1H, 5-H), 3.95 (s, 3H), 3.90 (s, 3H), 3.77 (m, 1H), 3.58 (s, 2H), 2.79 (d, 2H, *J*= 11.1 Hz), 2.57 (s, 3H, 3-Me), 2.08 (t, 2H, *J*= 11.5 Hz), 1.76 (m, 2H), 1.63 (m, 2H).

Preparation of 4,6-dihydroxy-3-methyl-*N*^{2}-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl) benzofuran-2,7-dicarboxamide (17b)

17b was synthesized by the method shown in **Scheme 3** as white solid with a yield of 4.0%. M.p.: 219.6-221.3°C, LC-MS: 492.2 $[M+H]^+$, 490.0 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 14.22 (s, 1H, 6-OH), 11.13 (s, 1H, 4-OH), 8.67 (d, 1H, *J*= 7.4 Hz), 8.22 (s, 1H, $CONH_2$), 7.98 (s, 1H, $CONH_2$), 7.86 (m, 3H), 7.75 (t, 1H, *J*= 7.8 Hz), 6.18 (s, 1H, 5-H), 4.43 (d, 2H, *J*= 4.4 Hz), 3.97 (m, 1H), 3.43 (d, 2H, *J*= 11.5 Hz), 3.13 (m, 2H), 2.60 (s, 3H, 3-Me), 2.07 (d, 2H, *J*= 13.6 Hz), 1.87 (m, 2H).

Preparation of 4,6-dimethoxy-3-methyl-*N*-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl) benzofuran-2-carboxamide (17c)

17c was synthesized by the method shown in **Scheme 4** as white solid with a yield of 40.0%. M.p.: 145.0-146.7°C, LC-MS: 477.2 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.10 (t, 1H, *J*= 7.4 Hz), 7.55-7.66 (m, 4H), 6.68 (d, 1H, *J*= 7.1 Hz), 6.41 (d, 1H, *J*= 1.6 Hz), 3.96 (d, 3H, *J*= 7.3 Hz), 3.84 (d, 3H, *J*= 19.0 Hz), 3.73-3.80 (m, 1H), 3.57 (m, 2H), 2.81 (d, 2H, *J*= 8.6 Hz), 2.59 (s, 3H, 3-Me), 2.06 (t, 2H, *J*= 10.7 Hz), 1.62-1.78 (m, 4H).

Preparation of 6-methoxy-3-methyl-*N*-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl)benzofuran-2-carboxamide (17d)

17d was synthesized by the method shown in **Scheme 5** as white solid with a yield of 35.0%. M.p.: 210.7-212.4°C, LC-MS: 447.1 $[M+H]^+$, 444.8 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.20 (d, 1H, J= 8.2 Hz), 7.58-7.66 (m, 5H), 7.10 (d, 1H, *J*= 2.0 Hz, 4-H), 6.96 (dd, 1H, *J*= 2.2 Hz, 8.6 Hz, 5-H), 3.83 (s, 3H, 6-OMe), 3.79 (m, 1H), 3.57 (s, 2H), 2.81 (d, 2H, *J*= 10.6 Hz), 2.48 (s, 3H, 3-Me), 2.06 (t, 2H, *J*= 12.0 Hz), 1.60-1.78 (m, 4H).

Preparation of 6-hydroxy-3-methyl-*N*-(1-(3-(trifluoromethyl)benzyl)piperidin-4-yl)benzofuran -2-carboxamide (17e)

17e was synthesized by the method shown in **Scheme 5** as white solid with a yield of 20.0%. M.p.: 114.4-116.2°C, LC-MS: 433.1 $[M+H]^+$, 430.9 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.87 (s, 1H, 6-OH), 8.17 (d, 1H, *J*= 8.2 Hz), 7.56-7.66 (m, 4H), 7.48 (d, 1H, *J*= 8.4 Hz), 6.89 (d, 1H, *J*= 1.7 Hz, 4-H), 6.80 (dd, 1H, *J*= 1.7 Hz, 8.4 Hz), 3.72-3.82 (m, 1H), 3.57 (s, 2H), 2.81 (d, 2H, *J*= 11.3 Hz), 2.46 (s, 3H, 3-Me), 2.06 (t, 2H, *J*= 10.7 Hz), 1.60-1.78 (m, 4H).

4.2 Biological assay

4.2.1. Mnks kinase assay

CGP-57380 was obtained from Selleck.cn as positive control in the biological evaluation assay, Ser/Thr KinEase assay kit (CisBio) was used to determine the Mnk1/2 kinases inhibition of the designed compounds using CGP-57380 as positive control. 0.99 ng of Mnk1 or Mnk2 was incubated with different concentrations of test compounds in an 10 μL reaction mixture (1 μM substrate S3, 392 μM ATP, 10 mM $MgCl_2$, 1 mM Dithiothreitol and 1× KinEASE enzymatic buffer) for 60 min at room temperature. Reactions were terminated through adding 5 μL SA-XL665 and 5 μL STK Ab detection reagents following the kit protocol. The ratio between the HTRF signals of 615 and 665 nm recorded with an Infinite® F500 microplate reader (Tecan, Switzerland) and IC_{50} values were calculated from the inhibition curves.

4.2.2. *In Vitro* Anti-proliferative Assay

The tested cells were plated in 96-well microtiter plates with a density of 2,000 cells/well and incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. The cells were then exposed to tested compounds of different concentrations in triplicate. After incubated for 96 h, 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide MTT solution (2 mg/mL) was added to each well and incubated for an additional 4 h. Then the suspernatant was discarded and the formazan was dissolved in 200 μL of dimethyl sulfoxide (DMSO). The plate was oscillated subtly at room temperature for 10 minutes before the absorbance was measured at 570 nm by a microplate reader. Cell inhibitory ratio was calculated with the following formula:

Inhibitory Rate (%) = [(Acontrol-Atreated)/Acontrol]×100%

The IC_{50} was determined as the concentration that caused half inhibition of cell proliferation. All experiments were performed three times independently, and the results were reported presented as mean.

4.2.3. Western blot analysis

The human colon cancer cell line HCT-116 cells were incubated in presence of the test compound **8k** (in 0.5% DMSO) for 24 h, harvested, and rinsed with ice-cold PBS. Total protein extracts (50 μg) were prepared by lysing cells in RIPA buffer [50 mM Tris-HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% NP-40, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 100 μM leupeptin, and 2 μg/mL aprotinin (pH 8.0)]. Samples were separated on an 8% or 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were stained with 0.2% Ponceau S red to assure equal protein loading and transfer. After blocking with 5% nonfat milk, the membranes were incubated with a specific antibody of eIF4E, p-eIF4E and GAPDH overnight at 4°C. Immunocomplexes were visualized using enhanced chemiluminescence western blotting detection reagents (Amersham Biosciences, England, and UK). Protein quantitation was determined by the Bradford protein binding assay.

4.3. Molecular modeling

Discovery studio 3.0 was used to perform in silico docking. The X-ray crystal structure of Mnk2 kinase in complex with staurosporine was retrieved from Protein Data Bank (PDB code 2HW7). All calculations and manipulations were performed with libdock modules in the Discovery studio software package. All water molecules were removed and hydrogen was added. Applying the default parameters, the best docking result was selected to analysis based on the favorable binding affinity rank in kcal/mol (docking score).

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Legends:

Figure 1. Chemical structures of known Mnks inhibitors.

Figure 2. Kinase inhibitors in development with carbamoyl moiety as hinge binder.

Figure 3. The structure of **FY0413** and the docking mode of **FY0413** in the ATP binding pocket of the Mnk2 kinase domain (**2HW7** in PDB). H-Bonding interactions were presented with green line.

Figure 4. **8k** decreased the expression of p-eIF4E in HCT-116 cells.

Figure 5. The proposed binding mode of **8k** in the ATP binding pocket of the Mnk2 kinase domain (**2HW7** in PDB). H-Bonding interactions were presented with green line.

Scheme 1. Synthesis of **5a-5r** and **FY0413**. Reagents and Conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone; (b) $BrCH_2COOC_2H_5$, K_2CO_3, DMF, 70-90°C; (c) CSI, CH_3CN, 0°C to r.t., then 1N HCl; (d) NaOH, CH_3OH/H_2O, 40°C; (e) RNH_2, HATU, DIEA, DMSO, r.t.; (f) BBr_3, CH_2Cl_2, -40°C to r.t.; (g) HBr(30%), CH_3COOH, reflux.

Scheme 2. Synthesis of **8a-8p** and **17a**. Reagents and Conditions: (a) 4-amino-1-Boc-piperidine or 4-aminomethyl-1-Boc-piperidine, HATU, DIEA, DMSO, r.t.; (b) HCl in ethyl acetate, r.t.; (c) substituted benzyl chloride or benzoyl chloride, K_2CO_3, DMF, 80°C; (d) BBr_3, CH_2Cl_2, -40°C to r.t..

Scheme 3. Synthesis of **17b.** Reagents and Conditions: (a) BBr_3, CH_2Cl_2, -40°C to r.t..

Scheme 4. Synthesis of **17c**. Reagents and Conditions: (a) NaOH, H_2O/CH_3OH, reflux; (b) 4-amino-1-Boc-piperidine, HATU, DIEA, DMSO, r.t.; (c) HCl in ethyl acetate, r.t.; (d) 3-trifluromethylbenzyl bromide, Cs2CO3, DMF, 80°C.

Scheme 5. Synthesis of **17d** and **17e**. Reagents and Conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone; (b) $BrCH_2COOC_2H_5$, K_2CO_3, DMF, 70-90°C; (c) NaOH, CH_3OH/H_2O, 40°C; (d) RNH_2, HATU, DIEA, DMSO, r.t.; (e) HCl in ethyl acetate, r.t.; (f) 3-trifluromethylbenzyl bromide, Cs_2CO_3, DMF. 80°C; (g) BBr_3, CH_2Cl_2, -40°C to r.t..

Table 1. The structure and inhibitory activities of compounds **FY0413** and **5a-5r** on Mnk2 kinase.

Table 2. The structure and inhibitory activities of compounds **8a-8p** on Mnk2.

Table 3. The structure and inhibitory activities of compounds **8k** and **17a-17e** on Mnk2.

Table 4. Inhibition effects of selected compounds towards Mnks and cancer cells.

Highlights

1. A novel series of 6-hydroxy-4-methoxy-3-methylbenzofuran-7-carboxamide derivatives featured with various C-2 substituents were designed and synthesized as Mnks inhibitors through fragment-based drug design.

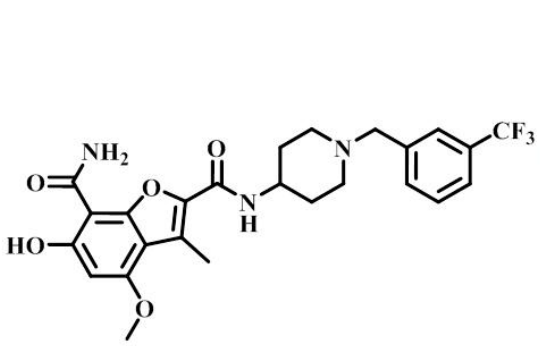
2. **8k** showed the most promising Mnks inhibitory activities.

3. **8k** exhibited strong anti-proliferative activity.

4. **8k** decreased the levels of phosphorylation of eIF4E at Ser209, but barely affected the levels of eIF4E.

4. Molecular docking studies demonstrated strong interactions between **8k** and Mnk2.

Graphical Abstract



8k

Mnk2: IC_{50}= 0.273 µM
Mnk1: %inhib. at 1 µM= 41%
THP-1 cells: IC_{50}= 6.30 µM
MOLM-13 cells: IC_{50}= 3.15 µM
HCT-116 cells: IC_{50}= 2.77 µM