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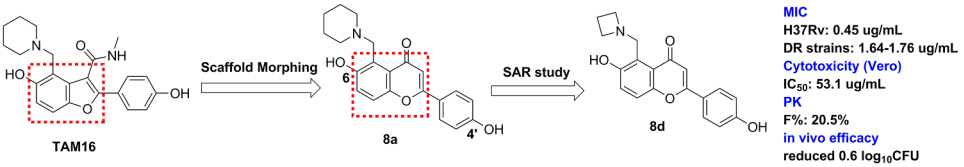

Design, synthesis, and biological evaluation of novel 4*H*-chromen-4-one derivatives as antituberculosis agents against multidrug-resistant tuberculosis

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Abstract

A series of 4*H*-chromen-4-one derivatives obtained by scaffold morphing of the benzofuran compound, TAM16, were tested for antitubercular activity. Compound **8d** was active against drug-sensitive and multidrug-resistant tuberculosis. A preliminary druggability evaluation showed that compound **8d** displayed favorable mouse and human microsomal stability, low cytotoxicity, and acceptable oral bioavailability. An *in vivo* study indicated that compound **8d** exhibited modest efficacy in an acute mouse model of TB after 3 weeks of treatment. Thus, **8d** is a promising antituberculosis lead compound.

Keywords: 4*H*-Chromen-4-one, Scaffold morphing, Multidrug-resistant tuberculosis, Polyketide synthase 13, Antitubercular agents

1. Introduction

Tuberculosis (TB) is one of the top 10 causes of death worldwide, and the lethality of TB is higher than that of HIV/AIDS. According to the WHO [1], in 2018, there were an estimated 10.0 million new cases of TB, around 1.2 million deaths due to TB, and around 0.25 million more deaths associated with HIV coinfected TB patients. Moreover, there were approximately 0.5 million new cases of rifampicin-resistant TB, of which 78% were multidrug-resistant TB [1]. Therefore, there is an urgent need for new anti-TB drugs.

The cell wall of *M. tuberculosis* are critical for its viability and virulence; therefore, cell wall synthesizing enzymes are attractive targets for anti-TB drug development [2]. Mycolic acids are long-chain fatty acids that are important for the mycobacterial cell wall permeability and integrity [3]. Disruption of the mycolic acids biosynthetic pathway is a strategy for anti-TB drug discovery that has been validated by many of the current first- and second-line anti-TB drugs, such as isoniazid, ethionamide, ethambutol, and clinical MmpL3 inhibitor SQ109 [4].

Polyketide synthase 13 (Pks13) performs the final assembly step in mycolic acids synthesis. Pks13 catalyzes a Claisen-type condensation between the carboxyacyl-CoA and meromycoloyl-AMP to produce a mycolic β-ketoester, and which linked through a thioester bond to the C-terminus (C-ACP) domain of Pks13. The thioesterase (TE) domain of Pks13 cleaves this thioester bond and forms an ester bond between the mycolic β-ketoester and Ser1533 of TE domain. Then, the TE domain of Pks13 transfers the condensation product onto trehalose to synthesize precursor of trehalose monomycolate (TMM). Therefore, inhibiting Pks13 interferes with the critical pathway of mycolic acids synthesis and kills *M. tuberculosis* [5-7]. Recently, a handful of Pks13 inhibitors have been reported (Fig. 1), and compounds TP2 (**1**) and TP4 (**2**) have minimum inhibitory concentrations (MICs) of 1.0 and 0.5 μM, respectively [8]. Compound **3** is a TP2 analogue that shows remarkable potency against *M. tuberculosis* H37Rv (MIC: 0.23 μM) and an impressive potency (MIC: 0.20–0.44 μM) against *M. tuberculosis* strains resistant to isoniazid, rifampicin, and fluoroquinolones [9]. β-Lactone compounds EZ120 (**4**) and orlistat (**5**) have been

identified as Pks13 inhibitors and EZ120 exhibited a MIC of 1.6 μM against *M. tuberculosis* H37Rv [10,11]. Benzofuran derivatives have also been identified as Pks13 inhibitors based on whole-cell screening and whole-genome sequencing of resistant mutants [12]. TAM16 (**6**; MIC: 0.09 μM) was developed by structure-guided optimization and is a promising candidate with potent efficacy in both acute and chronic mouse TB infection models and has favorable druggability [13]. Recently, Yu and coworkers reported coumestan compounds targeting Pks13, and compound **7** showed potent anti-TB activity in vitro (MIC: 0.0039 μg/mL) and in a serum inhibition titration assay [14,15]. Therefore, Pks13 is a promising druggable target for the development of new TB drugs.

Heterocycles containing N, O or S are valuable moieties for synthesizing many antitubercular agents, such as imidazo[1,2-*a*]pyridine [16], quinolone [17], thiazole [18], benzothiazinethione [19], pyridine [20], indole [21], 1,3,4-oxadiazole [22]. On the basis of the essentiality of Pks13 for survival of *M. tuberculosis* and the application of heterocyclic compounds in development of antitubercular agents, in this work, we developed anti-TB compounds by scaffold morphing of the benzofuran compound, TAM16. The heterocyclic compounds containing 4*H*-chromen-4-one scaffold exhibited good activity against *M. tuberculosis* H37Rv and drug-resistant TB isolates.

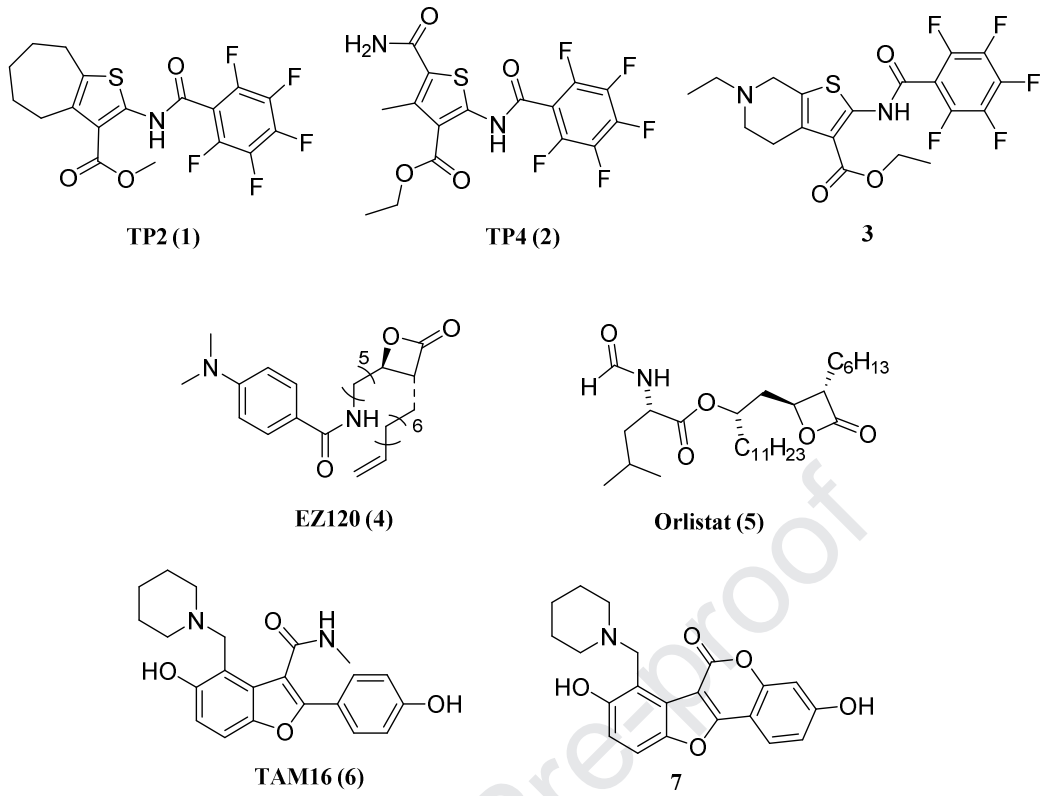
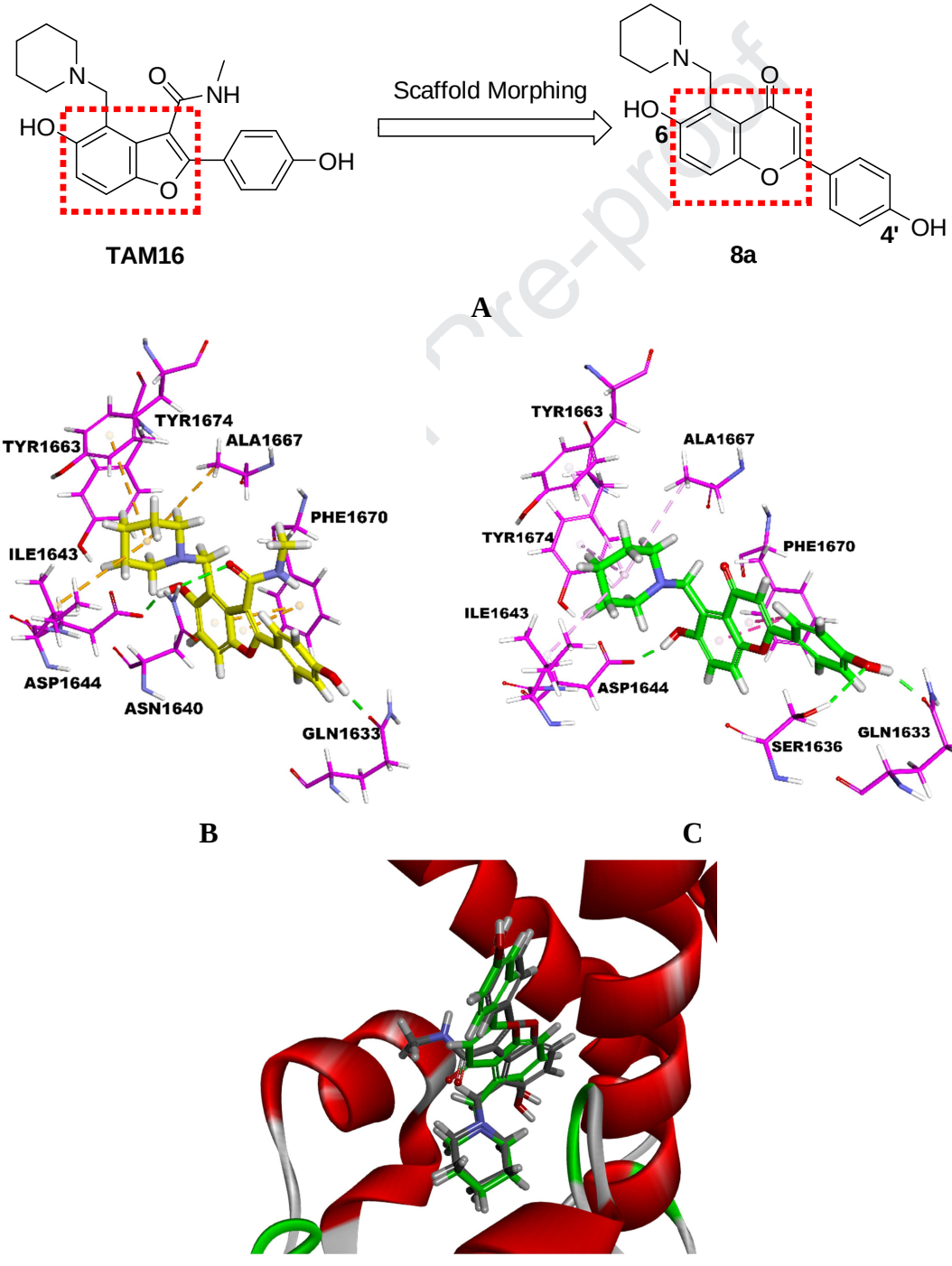


Fig. 1. Chemical structures of Pks13 inhibitors

2. Results and discussion

Our initial design was inspired by the Pks13 inhibitor TAM16. The crystal structure of TAM16 bound to Pks13 confirmed that the ligand blocks the active site of the Pks13-thioesterase (TE) domain [13]. Three hydrogen bonds were observed between the carbonyl oxygen of TAM16 and the amino hydrogen of Asn1640, and between the hydroxyls of TAM16 and the hydroxyl hydrogen of Asp1644 and the carbonyl oxygen of Gln1633. The piperidine ring of TAM16 formed van der Waals stacking interactions with residues such as Tyr1663. Guided by the crystal structure information, we used scaffold morphing to explore potential anti-TB agents containing the 4*H*-chromen-4-one frame (Fig. 2A). We made the key observation that compound **8a** adopts a similar conformation to TAM16 in the active pocket of the Pks13-TE domain (Fig. 2B, 2C and 2D). Moreover, the docking result showed that there were three hydrogen bond interactions, between 6-OH of **8a** and the hydroxyl hydrogen of Asp1644, and between 4'-OH of **8a** and the carbonyl oxygen of Gln1633

and the hydroxyl hydrogen of Ser1636. The piperidine ring of compound **8a** also formed van der Waals stacking interactions with residues such as Tyr1663 (Fig. 2C). Based on these results, we first synthesized and evaluated 4*H*-chromen-4-one **8a**. Compound **8a** displayed a moderate MIC (3.84 μg/mL) against *M. tuberculosis* H37Rv (Table 1), and this encouraged us to explore the systematic structure-activity relationship (SAR) against *M. tuberculosis* further to demonstrate the potential of the scaffold for hit-to-lead optimization.



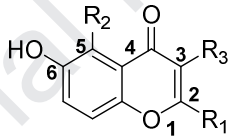
D

Fig. 2. (A) Design strategy of 4*H*-chromen-4-one-based anti-TB agent; (B) interaction of TAM16 bound to Pks13 (PDB code 5V3Y), TAM16 is shown in yellow, the hydrogen bonds are shown as green lines, and π–π stacking interactions are shown as orange lines; (C) predicted binding mode for **8a** with Pks13 (PDB code 5V3Y), compound **8a** is shown in green, the hydrogen bonds are shown as green lines, and π–π stacking interactions are shown as pink lines; (D) overlays of TAM16 and **8a** in the Pks13-TE domain, TAM16 is shown in gray, **8a** is shown in green.

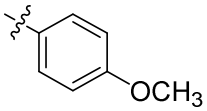
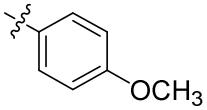
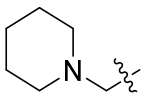
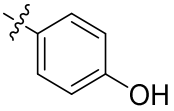
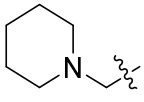
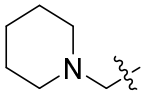
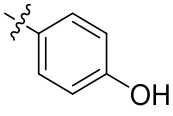
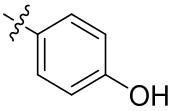
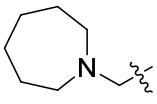
R_1, R_2, and R_3 in the 4*H*-chromen-4-ones were identified as sites for diversification (Table 1). When R_1 was 4-methoxyphenyl, compound **13a** displayed moderate anti-TB activity (MIC: 7.30 μg/mL); however, compound **12a** without substituent at R_2 did not show activity against *M. tuberculosis* (MIC: >32 μg/mL). This result demonstrated that the substituent at R_2 is an essential group for maintaining MIC and that 4-hydroxyphenyl at R_1 is preferred for cellular potency (compound **8a** vs compound **13a**). Switching the 4-hydroxyphenyl to the 3-position in compound **20** resulted in no activity compared with reference compound **8a**, indicating that the position of the 4-hydroxyphenyl had an important effect on activity, and that the 2-position was favored. Next, we explored the effect of the R_2 substituent on the potency by keeping a 4-hydroxyphenyl substituent at R_1. Azepan-1-yl-methyl (**8b**), pyrrolidin-1-yl-methyl (**8c**), and azetidine-1-yl-methyl (**8d**) R_2 substituents maintained the activity, and compound **8d** with a four-membered ring R_2 substituent showed the most potent activity (MIC: 0.45 μg/mL). In contrast, the ring-opened diethylamine and dimethylamine analogues **8e** and **8f**, respectively, had no activity. The addition of polar atoms to the piperidine ring (**8g**) removed the activity (MIC: >32 μg/mL). Compounds with 2-thia-6-azaspiro[3.3]heptanyl-1-methyl (**8h**) and cyclohexylaminomethyl (**8i**) groups showed weaker or no potency compared with compound **8a**. A small group at R_2 was favorable for activity, and the SAR trends we observed were different from those for benzofurans [13]. Next, we assessed the effects of substituting the benzene ring at the 2-position of the 4*H*-chromen-4-ones

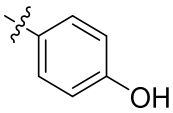
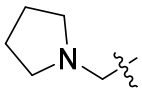
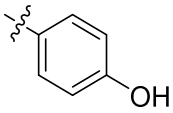
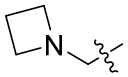
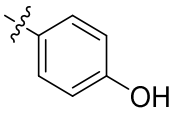
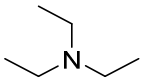
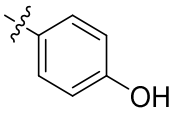
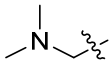
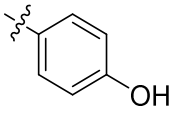
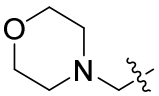
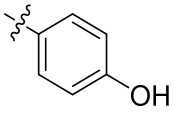
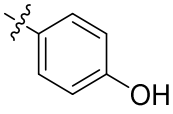
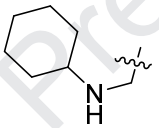
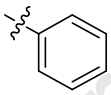
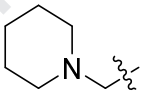
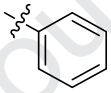
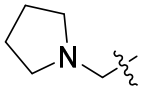
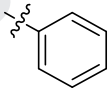
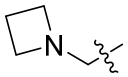
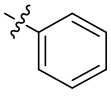
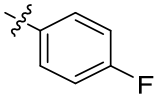
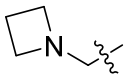
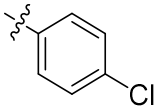
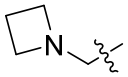
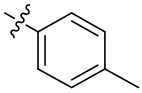
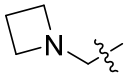
with H, an electron-donating Me group, and electron-withdrawing F and Cl groups. Although the compounds with these substituents (**13b**, **13e**, **13g**, **13h** and **13j**) showed modest activities, the hydroxyl substituent was the optimal group for anti-TB activity. Because there was a hydrogen bond between the carbonyl oxygen of TAM16 and the amino hydrogen of Asn1640, we investigated the introduction of ethyl ester and amide groups at the 3-position. These groups were less tolerated with activities ranging from 7.9 to >32 μg/mL. Small groups at R_2 were also favorable for activity in this series, which is consistent with the SAR trend above. As a result of their potency against *M. tuberculosis* H37Rv, the activities of compounds **8c** and **8d** against drug-resistant TB strains were investigated. All tested compounds displayed equivalent potency against 13946 and 14862 strains (extensively drug-resistant-TB) compared with the drug-sensitive strain (Table 2).

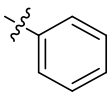
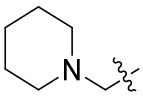
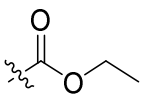
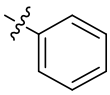
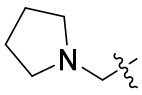
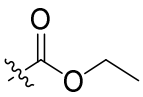
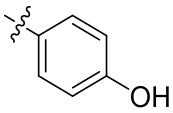
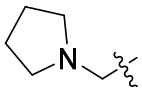
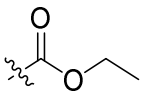
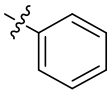
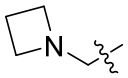
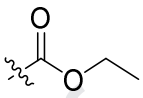
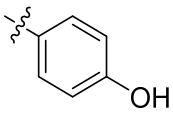
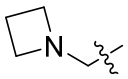
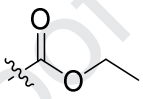
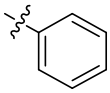
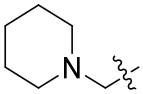
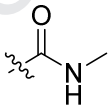
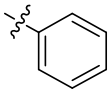
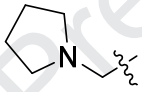
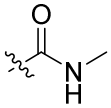
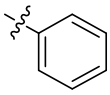
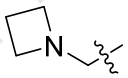
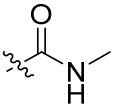
Table 1. Anti-TB activity of target compounds



8a-i, 12a, 13a-b, 13e, 13g-j, 13o
20, 29a-e, 30a-c

Compds.	R_1	R_2	R_3	MIC[a] (μg/mL)
12a		H	H	>32
13a			H	7.30
8a			H	3.84
20	H			>32
8b			H	7.20

8c			H	0.94
8d			H	0.45
8e			H	29.89
8f			H	>32
8g			H	>32
8h			H	15.34
8i			H	>32
13b			H	3.12
13e			H	3.68
13g			H	3.22
13o			H	31.11
13h			H	3.55
13i			H	28.54
13j			H	6.52

29a				31.85
29b				>32
29c				>32
29d				14.10
29e				7.91
32a				>32
32b				>32
32c				>32
TAM16				0.047
INH				0.039

[a] Minimum inhibitory concentration against *M. tuberculosis* H37Rv.

Table 2. *In vitro* activity of the selected compounds against drug-resistant TB strains

Compds.	MIC (μg/mL) ($H_{37}R_v$)	MIC (μg/mL) (13946[a])	MIC (μg/mL) (14862[b])
8c	0.938	3.806	3.818
8d	0.454	1.757	1.644
INH	0.039	2.379	>10
RFP	0.057	>10	>10

[a] Resistance to isoniazid (INH), streptomycin (SM), rifampicin (RFP), ethambutol (EMB), Rifabutin (RBT), *p*-aminosalicylate (PAS) and ofloxacin (OLFX). [b] Resistant to INH, SM, RFP, EMB, PAS, Prothionamide (1321) and Capreomycin (CPM).

The toxicities to Vero cells of compounds **8c** and **8d** (MIC: <1 μg/mL) were evaluated, and the selectivity index (SI) values were calculated from the ratio of IC_{50}/MIC (Table

3). Compound **8d** possessed low toxicity with high SI values. Furthermore, compounds **8c** and **8d** showed excellent metabolic stability against mouse and human liver microsomes (Table 3). Pharmacokinetic (PK) studies for compound **8d** were performed in BALB/c mice, following administration of a single oral dose and of an intravenous dose (Table 4). Compound **8d** exhibited favorable PK performance and a good PK profile with high plasma exposure ($AUC_{0-\infty}$ = 4286 ng·h/mL), and high maximal plasma concentration (C_{max} = 1656 ng/mL) after oral administration. Thus, compound **8d** had sufficiently promising PK properties to support *in vivo* efficacy studies in an animal model. The *in vivo* efficacy of compound **8d** was assessed in BALB/c mice in an acute TB infection model. Compound **8d** was orally administered at 100 mg/kg, whereas the positive control drug isoniazid (INH) was given at 25 mg/kg. The same formulation of 0.5% carboxymethylcellulose in water was used for both compounds. After three weeks of treatment, compound **8d** showed modest activity, reducing the bacterial burden in the lungs by 0.6 $\log_{10}$CFU compared with the untreated control group (Table 5).

Table 3. Cell toxicity, ClogP, and mouse liver microsome stability of selected compounds

Compds.	CLogP[a]	MIC (μg/mL)	Cytotoxicity IC_{50} (μg/mL)	SI[b]	Mouse microsome stability	
					Substrate remaining (%)[c]	Stability[d]
8c	2.49	0.938	8.8	9	102.4	stable
8d	2.26	0.454	53.1	117	96	stable

[a] CLogP was calculated using ADMET Predictor (TM) version 9.0.0.10, [b] SI = IC_{50}/MIC, [c] Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [d] Stability was determined without the NADPH cofactor.

Table 4. Mouse PK properties of compound **8d**

Parameters	unit	iv (5 mg/kg)	po (100 mg/kg)
$t_{1/2}$[a]	h	1.28	1.42
T_{max}	h	-	0.25
C_{max}/C_0	ng/mL	4459	1656
$AUC_{(0-t)}$[b]	h*ng/mL	1029	4223

$AUC_{(0-\infty)}$	h*ng/mL	1040	4286
$MRT_{(0-t)}$[c]	h	0.61	1.70
$MRT_{(0-\infty)}$	h	0.69	1.82
V	mL/kg	8856	-
CL	mL/h/kg	4806	-
F%[d]	-	-	20.5%

[a] Plasma elimination half-life, [b] Plasma exposure, [c] Mean residence time, [d] Oral bioavailability.

Table 5. Efficacy of compound **8d** after 21 days of treatment in BALB/c mice infected with *M. tuberculosis* $H_{37}R_v$ (mean ± SD)

Compds.	Dose (mg/kg)	Weight of mice (g)	Log_{10}CFU/lung
Control		21.32±1.03	5.35±0.17
8d	100	20.57±1.16	4.77±0.25
INH	25	20.90±0.95	1.76±0.49

Lastly, the binding mode of compound **8d** with PKs13 was investigated by using the CDOCKER protocol in Discovery Studio 2018 (Fig. 3). There were three hydrogen bond interactions, between the hydroxyl hydrogen of **8d** and the hydroxyl oxygen of ASP1644, the hydroxyl hydrogen of **8d** and the carbonyl oxygen of Gln1633, and the hydroxyl oxygen of compound **8d** and the hydroxyl hydrogen of Ser1636. The 4*H*-chromen-4-one scaffold of **8d** formed π-π interactions with Phe1670, and the azetidine ring of **8d** formed van der Waals stacking interactions with residues such as Tyr1663. This is also consistent with the proposed binding mode of the piperidine in TAM16. The interaction of the amide group oxygen of TAM16 with the side-chain amine of Asn1640 is proposed to be a key interaction; however, Asn1640 in the Pks13-TE domain had no obvious interactions with compound **8d**, which may be key for further structural modifications to increase the activity.

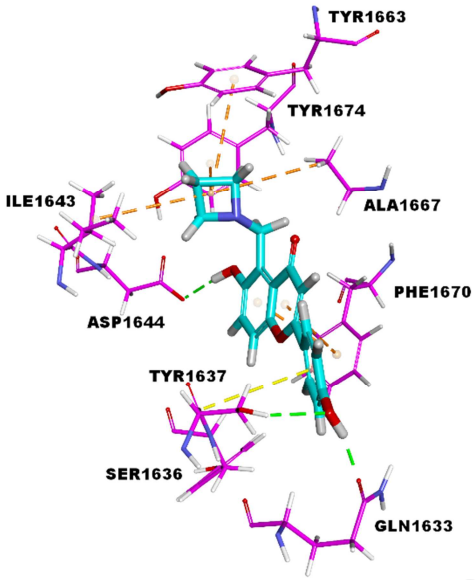
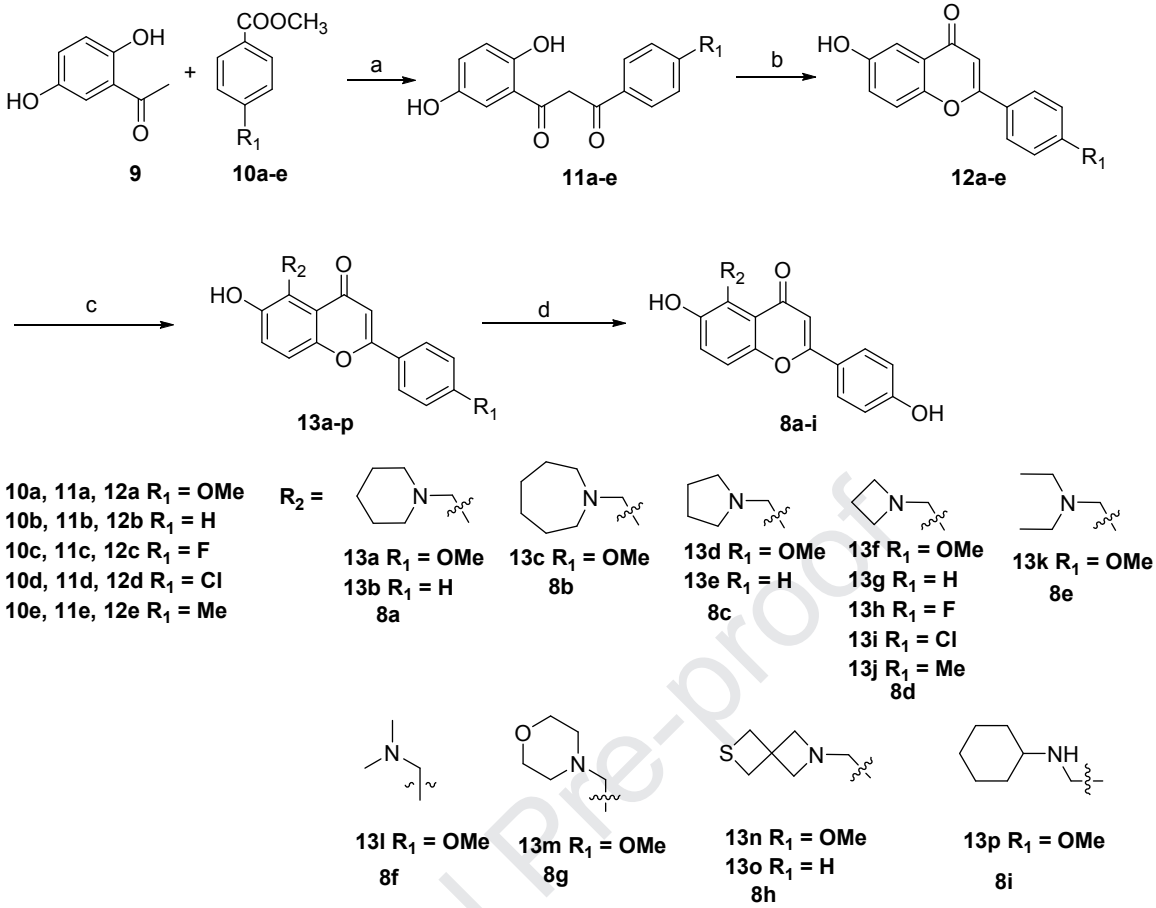


Fig. 3. Predicted binding mode of compound **8d** with PKs13 obtained from CDOCKER. The key amino acid residues are shown in purple in the active site of Pks13 (PDB ID 5V3Y). Compound **8d** is shown in cyan, the hydrogen bonds are shown as green lines, and the π–π stacking interactions are shown as orange lines.

3. Chemistry

Compounds **13a–p** and **8a–i** were synthesized in three or four steps using the synthetic routes shown in Scheme 1. Intermediates **11a–e** were prepared starting from 2,5-dihydroxyacetophenone (**9**) and methyl benzoate derivatives **10a–e** in the presence of lithium hexamethyldisilazide (LiHMDS). Compounds **12a–e** were obtained via cyclization of **11a–e** in the presence of concentrated sulfuric acid in glacial acetic acid at room temperature [23]. Mannich reaction of **12a–e** with 37% aqueous formaldehyde and the corresponding amine analogues afforded **13a–p** [24], when the amine was piperidine, the yields of Mannich reaction (**13a**,**b**) were lower than benzofuran system [13]. Pyrrolidine, azepane, azetidine, 2-thia-6-azaspiro[3.3]heptane, morpholine, diethylamine and dimethylamine were used as nucleophiles in Mannich reaction, the products were obtained in various yields (12-64%). Then, the compounds containing an OMe group were reacted with boron tribromide to give **8a–i**.

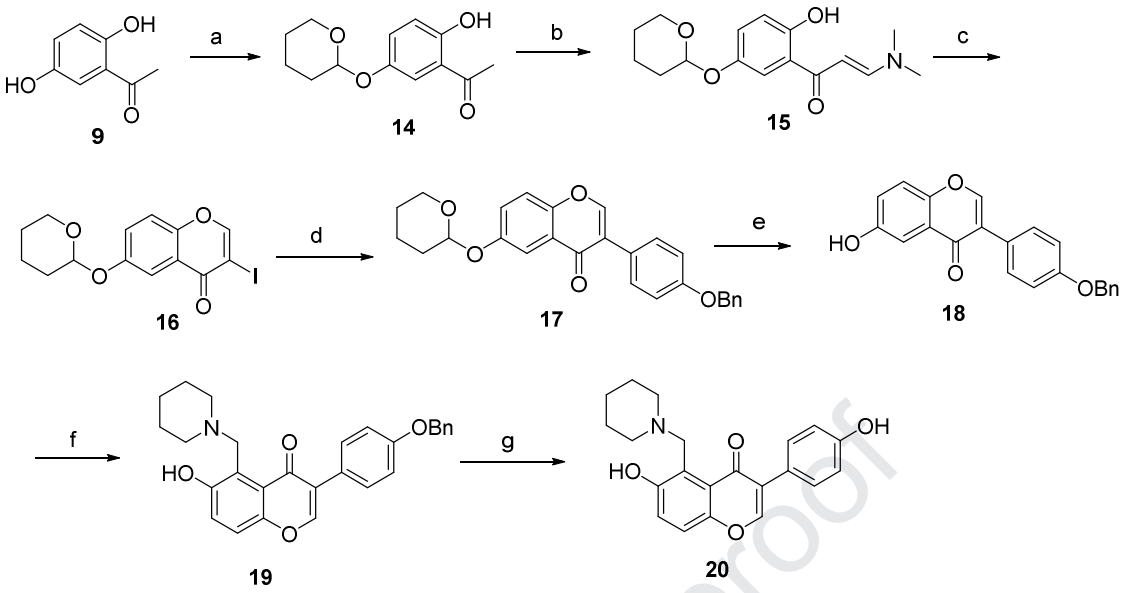


Scheme 1. Synthesis of compounds **8a-i**.

Reagent and conditions: (a) LiHMDS, THF, -78~10 °C, 3 h, -78 °C ~rt, overnight; (b) HOAc, H_2SO_4, rt, 4 h, 45-58%; (c) EtOH, reflux, 12-48 h, 12-64%; (d) BBr_3, CH_2Cl_2, rt, 5 h, 41-92%.

Compound **20** was synthesized from 2,5-dihydroxyacetophenone (**9**) via the synthetic route in Scheme 2. First, one hydroxyl in **9** was protected by tetrahydro-2*H*-pyran. Compound **14** was reacted with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) at 95 °C to afford compound **15**. Compound **16** was formed via cyclization of compound **15** in the presence of I_2 and pyridine at room temperature. Compound **16** served as a Suzuki coupling partner with (4-(benzyloxy)phenyl)boronic acid. Isoflavone derivative **17** was deprotected using *p*-toluenesulfonic acid to give hydroxyl compound **18** [25], which then underwent a Mannich reaction with 37% aqueous formaldehyde and piperidine to yield compound **19**. The benzyl protection of

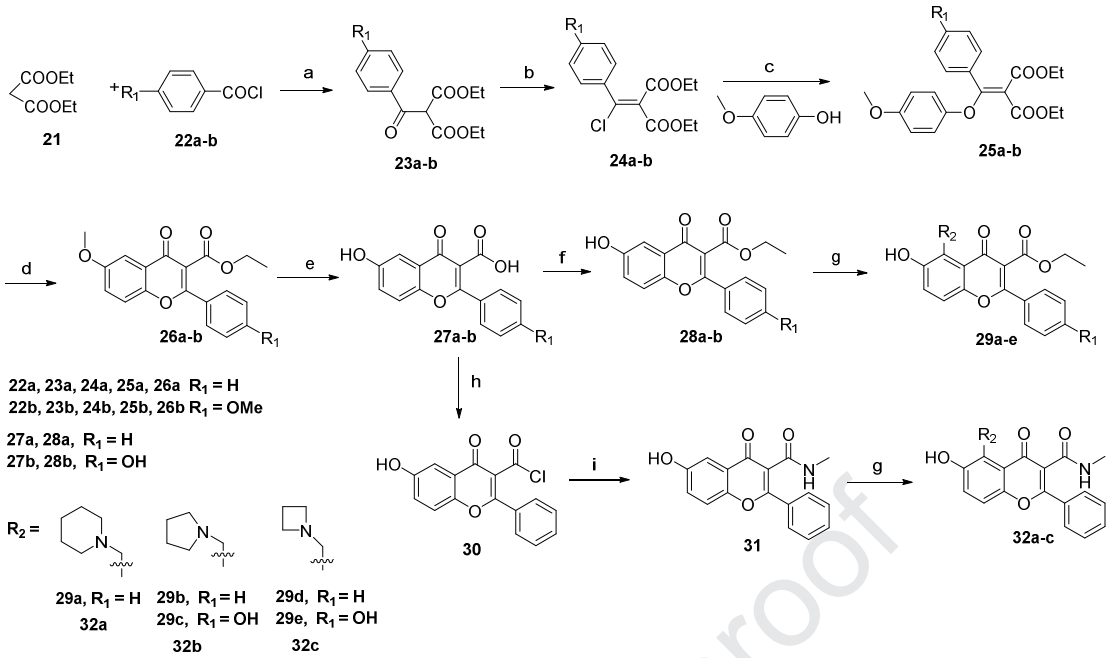
compound **19** was removed by catalytic hydrogenation to give target compound **20**.



Scheme 2. Synthesis of compound **20**.

Reagent and conditions: (a) *p*-TsOH-Py, CH_2Cl_2, rt, 5 h, 87%; (b) DMF-DMA, 95 °C, 5 h, 50%; (c) I_2, Py, $CHCl_3$, rt, 7 h, 79%; (d) Pd/C, $NaCO_3$, DME-H_2O, 45 °C, 4 h, 57%; (e) *p*-TsOH, MeOH-THF, 60 °C, 2 h, 64%; (f) HCHO, Piperidine, MeOH, reflux, 48 h, 46%; (g) H_2, Raney Ni, rt, 2 h, 37%.

Compounds **25a,b** were prepared following the procedure described in the literature from diethyl malonate and benzoyl chloride [26,27]. Compounds **26a,b** were constructed by cyclization of compounds **25a,b** in the presence of polyphosphoric acid [27]. Hydroxyl compounds **28a,b** were formed from compounds **26a,b** via sequential demethylation and esterification using boron tribromide and EtOH/H_2SO_4, respectively, and then **28a,b** underwent a Mannich reaction with 37% aqueous formaldehyde and corresponding amines to yield target compounds **29a–e**. Amides **32a–c** were synthesized from intermediates **27a,b** (Scheme 3).



Scheme 3. Synthesis of compounds **29a-e** and **32a-c**.

Reagent and conditions: (a) $MgCl_2$, TEA, CH_3CN, 0-rt, 5 h; (b) $POCl_3$, TEA, 110 °C, 6 h; (c) K_2CO_3, DMF, 140, 6 h; (d) PPA, 100 °C, 4 h, four steps, 30-33%; (e) BBr_3, CH_2Cl_2, rt, 5 h; (f) H_2SO_4, EtOH, reflux, 12 h, two steps, 88-95%; (g) EtOH, reflux, 12-48 h, 34-57%; (h) $(COCl)_2$, cat.DMF, CH_2Cl_2, rt, 4 h; (i) NH_2CH_3, THF, rt, overnight, two steps, 24%.

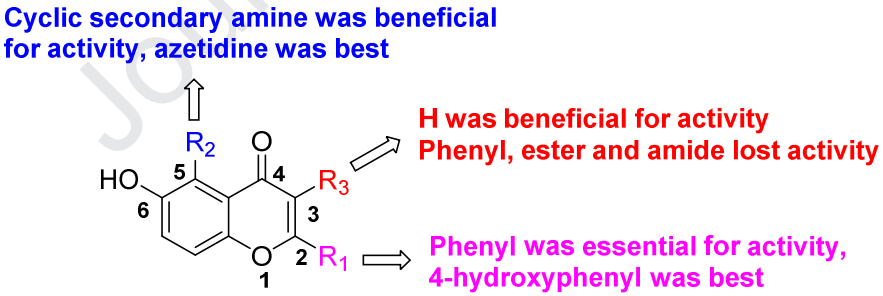


Fig. 4. Structure-activity relationships of 4*H*-chromen-4-ones.

4. Conclusion

4*H*-Chromen-4-one derivatives were synthesized by scaffold morphing from benzofurans and their anti-TB activities were tested. The SAR studies demonstrated that the aryl group at the 2-position was essential for activity, and the 4-hydroxyphenyl substituent was favorable for activity (Fig. 4). At the 3-position of the 4*H*-chromen-4-one frame, hydrogen gave the best activity. Compounds with a

small secondary-amine ring at the 5-position showed potent activities against *M. tuberculosis*. The docking study revealed key hydrogen bond interactions of compound **8d** with Asp1644, Gln1633, and Ser1636 residues of Pks13. Importantly, **8d** was active against multidrug-resistant TB isolates. Preliminary druggability evaluation of this compound revealed favorable mouse and human microsomal stability, low cytotoxicity, and acceptable oral bioavailability. Furthermore, an in vivo study indicated that compound **8d** exhibited moderate efficacy in an acute mouse model of TB after 3 weeks of treatment. Hence, compound **8d** is a promising anti-TB lead compound warranting further optimization, which will be reported in due course.

5. Experimental

5.1. General information

All the solvents and chemical reagents were purchased from commercial sources and used without further purification. TLC was performed on silica gel plates (GF254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (300-400 mesh). ^{1}H NMR spectra were obtained on Varian Mercury at 400 MHz or 300 MHz. ^{13}C NMR spectra were obtained on Varian Mercury at 100 MHz or 125 MHz. $CDCl_3$, DMSO-d_6 or CD_3OD were used as the NMR solvents and tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) values were referenced to the residual solvent peak and reported in ppm and all coupling constant (*J*) values were given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, and (brs) broad. HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Melting points were determined on Yanaco MP-J3 microscope melting point apparatus.

*5.2. General procedure for the synthesis of **12a–e***

A solution of LiHMDS in THF (1 M, 4 equiv) was added to a well-stirred solution of 2,5-dihydroxyacetophenone (**9**, 1 equiv) in anhydrous THF (20 mL) under argon at

-78 °C over 15 min. The reaction mixture was stirred at -78 °C for 1 h and at -10 °C for 2 h and was cooled again to -78 °C, and a solution of **10a–e** (1 equiv) in THF (5 mL) was added in one portion. The mixture was stirred at -78 °C for 1 h and at room temperature overnight. The reaction mixture was poured into a mixture of ice and neutralized with conc. HCl (6.5 mL) and extracted with dichloromethane. The combined organic layer was washed with brine and dried over sodium sulfate, filtered, and evaporated to give crude **11a–e**. Crude **11a–e** were dissolved in glacial acetic acid (20 mL) and concentrated sulfuric acid (0.2 mL). The reaction was stirred at room temperature for 4 h. H_2O (20 mL) was added to the reaction mixture, and the resulting solid was filtered, washed with water, and dried to afford **12a–e**.

*5.2.1. 6-Hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**12a**).*

The title compound was synthesized from 2,5-dihydroxyacetophenone **9** (10 mmol, 1.52 g) and methyl *p*-methoxybenzoate **10a** (10 mmol, 1.66 g) following the typical procedure. Brown solid (1.47 g, 55%). mp: 136-138 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 9.2 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 3.2 Hz, 1H), 7.24 (dd, *J* = 8.8, 3.2 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 6.87 (s, 1H), 3.86 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.51, 162.91, 162.63, 155.43, 149.92, 128.72, 124.84, 124.12, 123.49, 120.39, 115.18, 108.15, 105.14, 56.16. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{13}O_4$: 269.0808, found: 269.0804.

*5.2.2. 6-Hydroxy-2-phenyl-4H-chromen-4-one (**12b**).*

The title compound was synthesized from 2,5-dihydroxyacetophenone **9** (10 mmol, 1.52 g) and methyl benzoate **10b** (10 mmol, 1.26 mL) following the typical procedure. Brown solid (1.07 g, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.10-8.08 (m, 2H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.60-7.58 (m, 3H), 7.33 (d, *J* = 2.8 Hz, 1H), 7.27 (dd, *J* =8.8, 2.8 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.47, 162.65, 155.38, 149.86, 132.10, 131.86, 129.57, 126.71, 124.72, 123.57, 120.33, 107.96, 106.41.

*5.2.3. 6-Hydroxy-2-(4-fluorophenyl)-4H-chromen-4-one (**12c**).*

The title compound was synthesized from 2,5-dihydroxyacetophenone **9** (5 mmol, 761 mg) and methyl 4-fluorobenzoate **10c** (5 mmol, 771 mg) following the typical procedure. Gray solid (0.62 g, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.18-8.15 (m, 2H), 7.66 (d, J = 8.8 Hz, 1H), 7.45-7.40 (m, 2H), 7.32 (d, J = 3.2 Hz, 1H), 7.26 (dd, J = 8.8, 3.2 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.42, 164.56 (d, J_{C-F} = 248.5), 161.71, 155.39, 149.80, 129.37 (d, J_{C-F} = 8.9), 128.41 (d, J_{C-F} = 2.9), 124.62, 123.56, 120.30, 116.65 (d, J_{C-F} = 21.9), 107.96, 106.32.

*5.2.4. 6-Hydroxy-2-(4-chlorophenyl)-4H-chromen-4-one (**12d**).*

The title compound was synthesized from 2,5-dihydroxyacetophenone **9** (5 mmol, 761 mg) and methyl 4-chlorobenzoate **10d** (5 mmol, 853 mg) following the typical procedure. Gray solid (0.68 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.12 (d, J = 8.4 Hz, 2H), 7.67-7.64 (m, 3H), 7.32 (d, J = 2.8 Hz, 1H), 7.27 (dd, J = 8.8, 2.8 Hz, 1H), 7.00 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.43, 161.48, 155.44, 149.80, 136.88, 130.76, 129.65, 128.55, 124.68, 123.67, 120.36, 107.94, 106.70.

*5.2.5. 6-Hydroxy-2-(4-methylphenyl)-4H-chromen-4-one (**12e**).*

The title compound was synthesized from 2,5-dihydroxyacetophenone **9** (5 mmol, 761 mg) and methyl 4-methylbenzoate **10e** (5 mmol, 751 mg) following the typical procedure. Brown solid (0.73 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.8 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 3.2 Hz, 1H), 7.26 (dd, J = 8.8, 3.2 Hz, 1H), 6.91 (s, 1H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.41, 162.79, 155.32, 149.80, 142.27, 130.15, 129.03, 126.63, 124.72, 123.45, 120.26, 107.98, 105.77, 21.50.

*5.3. General procedure for the synthesis of **13a–p***

Formalin (37%, 1.5 equiv) and amine derivatives (1.5 equiv) were added to a solution of **12a–e** in ethanol (15 mL). The reaction mixture was stirred for 12–48 h under argon at 80 °C. The reaction solution was then cooled to room temperature and

concentrated in vacuo. The residue was purified by column chromatography to give compounds **13a–p**.

*5.3.1. 6-Hydroxy-2-(4-methoxyphenyl)-5-(piperidin-1-ylmethyl)-4H-chromen-4-one (**13a**).*

The title compound was prepared from compound **12a** (0.66 mmol, 178 mg), formalin (37%, 1 mmol, 81 μL) and piperidine (1 mmol, 99 μL) following the typical procedure. Pale yellow solid (90 mg, 37%). mp: 187-188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, J = 9.2 Hz, 2H), 7.57 (d, J = 8.8 Hz, 1H), 7.15-7.10 (m, 3H), 6.78 (s, 1H), 4.63 (s, 2H), 3.85 (s, 3H), 2.54 (brs, 4H), 1.60-1.46 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.01, 162.43, 161.11, 156.97, 150.67, 128.44, 123.52, 123.16, 122.35, 119.12, 118.58, 115.01, 106.29, 56.92, 55.98, 53.53, 25.60, 23.58. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{24}O_4N$: 366.1700, found: 366.1708.

*5.3.2. 6-Hydroxy-2-phenyl-5-(piperidin-1-ylmethyl)-4H-chromen-4-one (**13b**).*

The title compound was synthesized from compound **12b** (1.10 mmol, 261 mg), formalin (37%, 1.64 mmol, 133 μL) and piperidine (1.64 mmol, 163 μL) following the typical procedure. Yellow solid (104 mg, 28%). mp: 178-179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (dd, J =8.0, 1.6 Hz, 2H), 7.60-7.57 (m, 4H), 7.16 (d, J = 9.2 Hz, 1H), 6.88 (s, 1H), 4.62 (s, 2H), 2.51 (brs, 4H), 1.58-1.46 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.32, 161.09, 157.39, 150.95, 132.25, 131.61, 129.76, 126.79, 123.61, 122.54, 119.23, 119.07, 107.85, 57.47, 53.75, 25.98, 23.89. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{22}O_3N$: 336.1594, found: 336.1604.

*5.3.3. 6-Hydroxy-2-phenyl-5-(pyrrolidin-1-ylmethyl)-4H-chromen-4-one (**13e**).*

The title compound was synthesized from compound **12b** (0.91 mmol, 217 mg), formalin (37%, 1.36 mmol, 110 μL) and pyrrolidine (1.36 mmol, 112 μL) following the typical procedure. Yellow solid (101 mg, 23%). mp: 133-135 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08-8.06 (m, 2H), 7.61-7.57 (m, 4H), 7.17 (d, J = 9.2 Hz, 1H), 6.88 (s, 1H), 4.76 (s, 2H), 2.68-2.63 (m, 4H), 1.82-1.78 (m, 4H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 180.09, 160.95, 157.19, 150.65, 132.06, 131.46, 129.57, 126.61, 123.36, 122.00, 119.68, 119.07, 107.68, 54.06, 53.38, 23.73. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{20}O_3N$: 322.1438, found: 322.1448.

*5.3.4. 5-(Azetidin-1-ylmethyl)-6-hydroxy-2-phenyl-4H-chromen-4-one (**13g**).*

The title compound was synthesized from compound **12b** (0.42 mmol, 100 mg), formalin (37%, 0.63 mmol, 51 μL) and azetidine (0.63 mmol, 42 μL) following the typical procedure. Brown solid (68 mg, 53%). mp: 130-133 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (d, J = 6.5 Hz, 2H), 7.58 (brs, 4H), 7.17 (d, J = 9.0 Hz, 1H), 6.88 (s, 1H), 4.69 (s, 2H), 3.34 (t, J = 7.5 Hz, 4H), 2.14-2.08 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.01, 160.96, 156.85, 150.69, 132.03, 131.44, 129.56, 126.58, 123.47, 122.26, 119.19, 118.74, 107.66, 56.87, 54.61, 17.24. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{18}O_3N$: 308.1281, found: 308.1278.

*5.3.5. 5-(Azetidin-1-ylmethyl)-2-(4-fluorophenyl)-6-hydroxy-4H-chromen-4-one (**13h**).*

The title compound was synthesized from compound **12c** (0.59 mmol, 150 mg), formalin (37%, 0.88 mmol, 71 μL) and azetidine (0.88 mmol, 59 μL) following the procedure. Pale yellow solid (75 mg, 39%). mp: 189-192 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16-8.12 (m, 2H), 7.58 (d, J = 9.2 Hz, 1H), 7.43-7.39(m, 2H), 7.17 (d, J = 9.2 Hz, 1H), 6.89 (s, 1H), 4.68 (s, 2H), 3.35 (t, J = 7.2 Hz, 4H), 2.15-2.08 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.94, 164.47 (d, J = 248.4 Hz), 160.02, 156.88, 150.63, 129.23 (d, J = 8.9 Hz), 128.00 (d, J = 2.9 Hz), 123.44, 122.14, 119.12, 118.81, 116.63 (d, J = 21.9 Hz), 107.57, 56.96, 54.61, 17.25. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{17}O_3NF$: 326.1187, found: 326.1183.

*5.3.6. 5-(Azetidin-1-ylmethyl)-2-(4-chlorophenyl)-6-hydroxy-4H-chromen-4-one (**13i**).*

The title compound was synthesized from compound **12d** (0.55 mmol, 150 mg), formalin (37%, 0.83 mmol, 67 μL) and azetidine (0.83 mmol, 56 μL) following the typical procedure. Pale yellow solid (22 mg, 12%). mp: 197-199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8

Hz, 1H), 7.17 (d, *J* = 9.2 Hz, 1H), 6.92 (s, 1H), 4.67 (s, 2H), 3.33 (t, *J* = 7.2 Hz, 4H), 2.14-2.07 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.95, 159.85, 156.86, 150.64, 136.82, 130.35, 129.64, 128.44, 123.55, 122.24, 119.27, 118.65, 107.97, 56.68, 54.62, 17.23. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{17}O_3NCl$: 342.0892, found: 342.0886.

*5.3.7. 5-(Azetidin-1-ylmethyl)-2-(4-methylophenyl)-6-hydroxy-4H-chromen-4-one (**13j**).*

The title compound was synthesized from compound **12e** (0.6 mmol, 150 mg), formalin (37%, 0.89 mmol, 72 μL) and azetidine (0.89 mmol, 60 μL) following the typical procedure. Yellow solid (65 mg, 34%). mp: 149-151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* =8.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.18 (d, *J* = 9.2 Hz, 1H), 6.86 (s, 1H), 4.69 (s, 2H), 3.38 (t, *J* = 7.2 Hz, 4H), 2.39 (s, 3H), 2.16-2.08 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.96, 161.19, 156.68, 150.64, 142.23, 130.14, 128.59, 126.53, 123.35, 122.28, 119.30, 118.44, 107.06, 56.45, 54.61, 21.50, 17.20. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{20}O_3N$: 322.1438, found: 322.1433.

*5.3.8. 5-((2-Thia-6-azaspiro[3.3]heptan-6-yl)methyl)-6-hydroxy-2-phenyl-4H-chromen-4-one (**13o**).*

The title compound was synthesized from compound **12b** (0.94 mmol, 224 mg), formalin (37%, 1.40 mmol, 114 μL) and 2-thia-6-azaspiro[3.3]heptane (1.40 mmol, 162 mg) following the typical procedure. Off-white solid (80 mg, 23%). mp: 207-210 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08-8.06 (m, 2H), 7.62-7.57 (m, 4H), 7.20 (d, *J* = 8.8 Hz, 1H), 6.88 (s, 1H), 4.63 (s, 2H), 3.39 (s, 4H), 3.30 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.97, 161.12, 156.13, 150.85, 132.12, 131.39, 129.59, 126.63, 123.40, 122.37, 119.54, 118.61, 107.71, 66.71, 54.99, 43.32, 36.13. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{20}O_3NS$: 366.1158, found: 366.1153.

*5.4. General procedure for the synthesis of **8a–i***

Boron tribromide (3 equiv) was added to a solution of corresponding compound **13** (1 equiv) in dichloromethane (10 mL) at -78 °C under argon and the mixture was stirred for 5 h at room temperature. The reaction was quenched with methanol and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give compounds **8a–i**.

*5.4.1. 6-Hydroxy-2-(4-hydroxyphenyl)-5-(piperidin-1-ylmethyl)-4H-chromen-4-one (**8a**).*

The title compound was synthesized from compound **13a** (0.25 mmol, 90 mg) following the typical procedure. Yellow solid (80 mg, 92%). mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 9.2 Hz, 1H), 7.46 (d, *J* = 9.2 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.90 (s, 1H), 4.70 (d, *J* = 4.8 Hz , 2H), 3.21-3.12 (m, 4H), 1.87-1.64 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.24, 162.91, 161.79, 154.87, 150.94, 129.02, 123.44, 123.26, 122.33, 121.68, 116.69, 113.07, 106.03, 53.23, 51.68, 22.76, 21.75. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{22}O_4N$: 352.1543, found: 352.1550.

*5.4.2. 5-(Azepan-1-ylmethyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8b**).*

Compound **13c** was synthesized from compound **12a** (0.62 mmol, 167 mg), formalin (37%, 0.93 mmol, 76 μL) and azepane (0.93 mmol, 105 μL) following the procedure described in *5.3*. Yellow solid (58 mg, 25%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, *J* = 9.2 Hz, 2H), 7.59 (d, *J* = 9.2 Hz, 1H), 7.17 (d, *J* = 9.2 Hz, 1H), 7.11 (d, *J* = 9.2 Hz, 2H), 6.79 (s, 1H), 4.76 (s, 2H), 3.86 (s, 3H), 2.78 (t, *J* = 5.6 Hz , 4H), 1.66-1.59 (m, 8H).

The title compound was synthesized from compound **13c**（0.15 mmol, 58 mg） following the typical procedure. Yellow solid (50 mg, 89%). mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 9.2 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.89 (s, 1H), 4.71 (s, 2H), 3.26 (brs, 4H), 1.83 (brs, 4H), 1.63 (brs, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.12, 162.75, 161.62, 155.14, 150.80, 128.83, 123.26, 122.99, 121.74, 121.51, 116.49,

114.15, 105.76, 54.25, 51.94, 27.27, 23.38. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{24}O_4N$: 366.1700, found: 366.1689.

*5.4.3. 6-Hydroxy-2-(4-hydroxyphenyl)-5-(pyrrolidin-1-ylmethyl)-4H-chromen-4-one (**8c**).*

Compound **13d** was synthesized from compound **12a** (1.03 mmol, 275 mg), formalin (37%, 1.54 mmol, 115 μL) and tetrahydropyrrole (1.54 mmol, 126 μL) following the procedure described in *5.3*. Yellow solid (180 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.16 (d, *J* = 8.8Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.60 (s, 1H), 4.91 (s, 2H), 3.89 (s, 3H), 2.78 (s, 4H), 1.91-1.88 (m, 4H).

The title compound was synthesized from compound **13d** (0.51 mmol, 180 mg) following the typical procedure. Yellow solid (122 mg, 71%). mp: 142-144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* =8.8 Hz, 1H), 7.12 (d, *J* = 9.2 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.69 (s, 1H), 4.76 (s, 2H), 2.64 (s, 4H), 1.80 (s, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.94, 161.54, 161.35, 157.08, 150.45, 128.52, 123.02, 121.89, 121.75, 119.50, 118.88, 116.40, 105.61, 53.97, 53.36, 23.70. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{20}O_4N$: 338.1387, found: 338.1394.

*5.4.4. 5-(Azetidin-1-ylmethyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8d**).*

Compound **13f** was synthesized from compound **12a** (0.75 mmol, 200 mg), formalin (37%, 1.12 mmol, 91 μL) and azetidine (1.12 mmol, 75 μL) following the procedure described in *5.3*. Pale yellow solid (85 mg, 34%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.03 (d, *J* = 8.5 Hz, 2H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.17 (d, *J* = 9.0 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.80 (s, 1H), 4.70 (s, 2H), 3.85 (s, 3H), 3.40 (s, 4H), 2.18-2.07 (m, 2H).

The title compound was synthesized from compound **13f** (0.25 mmol, 85 mg) following the typical procedure. Yellow solid (64 mg, 79%). mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.14 (d, *J*

= 8.8 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.71 (s, 1H), 4.70 (s, 2H), 3.36 (t, *J* = 7.2 Hz, 4H), 2.15-2.08 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.84, 161.77, 161.27, 156.28, 150.59, 128.60, 123.12, 122.28, 121.80, 119.60, 117.71, 116.38, 105.69, 55.35, 54.63, 17.08. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{18}O_4N$: 324.1230, found: 324.1228.

*5.4.5. 5-((Diethylamino)methyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8e**).*

Compound **13k** was synthesized from compound **12a** (0.78 mmol, 210 mg), formalin (37%, 1.18 mmol, 95 μL) and diethylamine (1.18 mmol, 121 μL) following the procedure described in *5.3*. Pale yellow solid (44 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.13-7.10 (m, 3H), 6.78 (s, 1H), 4.72 (s, 2H), 3.86 (s, 3H), 2.64 (q, *J* = 7.2 Hz, 4H), 1.07 (t, *J* = 7.2 Hz, 6H).

The title compound was synthesized from compound **13k** (0.12 mmol, 44 mg) following the typical procedure. Yellow solid (30 mg, 71%). mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.68 (s, 1H), 4.71 (s, 2H), 2.61 (d, *J* = 7.2 Hz, 4H), 1.06 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.03, 161.45, 161.23, 157.52, 150.54, 128.54, 123.08, 122.16, 121.83, 119.39, 118.83, 116.36, 105.67, 52.36, 46.60, 11.30. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{22}O_4N$: 340.1543, found: 340.1552.

*5.4.6. 5-((Dimethylamino)methyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8f**).*

Compound **13l** was synthesized from compound **12a** (0.29 mmol, 78 mg), formalin (37%, 0.44 mmol, 35 μL) and dimethylamine (0.44 mmol, 218 μL) following the procedure described in *5.3*. Yellow solid (58 mg, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, *J* = 9.2 Hz, 2H), 7.58 (d, *J* = 9.2 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 9.2 Hz, 2H), 6.79 (s, 1H), 4.61 (s, 2H), 3.86 (s, 3H), 2.32 (s, 6H).

The title compound was synthesized from compound **13l** (0.18 mmol, 58 mg) following the typical procedure. Yellow solid (25 mg, 45%). mp: 184-187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 9.2 Hz, 1H), 7.20 (d, *J* = 9.2 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.73 (s, 1H), 4.61 (s, 2H), 2.42 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.11, 161.97, 161.48, 156.77, 150.80, 128.81, 123.26, 122.52, 121.96, 119.89, 118.11, 116.57, 105.88, 56.82, 44.28. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{18}O_4N$: 312.1230, found: 312.1237.

*5.4.7. 6-Hydroxy-2-(4-hydroxyphenyl)-5-(morpholinomethyl)-4H-chromen-4-one (**8g**).*

Compound **13m** was synthesized from compound **12a** (0.72 mmol, 192 mg), formalin (37%, 1.08 mmol, 80 μL) and morpholine (1.08 mmol, 94 μL) following the procedure described in *5.3*. Off-white solid (168 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 9.0Hz, 1H), 7.18 (d, *J* =9.0 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.61 (s, 1H), 4.81 (s, 2H), 3.89 (s, 3H), 3.80 (brs, 4H), 2.71 (brs, 4H).

The title compound was synthesized from compound **13m** (0.46 mmol, 168 mg) following the typical procedure. Yellow solid (49 mg, 34%). mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* =9.2 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.70 (s, 1H), 4.62 (s, 2H), 3.62 (s, 4H), 2.53 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.03, 161.64, 161.39, 156.24, 151.08, 128.75, 123.16, 122.62, 122.02, 119.33, 119.16, 116.54, 105.98, 66.68, 56.19, 53.14. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{20}O_5N$: 354.1336, found: 354.1327.

*5.4.8. 5-((2-Thia-6-azaspiro[3.3]heptan-6-yl)methyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8h**).*

Compound **13n** was synthesized from compound **12a** (1.23 mmol, 330 mg), formalin (37%, 1.85 mmol, 150 μL) and 2-thia-6-azaspiro[3.3]heptane (1.85 mmol, 213 mg) following the procedure described in *5.3*. Yellow solid (150 mg, 31%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.03 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 9.0

Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.81 (s, 1H), 4.67 (s, 2H), 3.86 (s, 3H), 3.53 (s, 4H), 3.38 (s, 4H).

The title compound was synthesized from compound **13n** (0.38 mmol, 150 mg) following the typical procedure. Yellow solid (60 mg, 41%). mp: >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 9.0 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.70 (s, 1H), 4.63 (s, 2H), 3.37 (s, 4H), 3.30 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.77, 161.74, 161.27, 155.84, 150.70, 128.60, 123.03, 122.32, 121.80, 119.60, 116.38, 105.73, 66.62, 54.49, 43.23, 36.11. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{20}O_4NS$: 382.1108, found: 382.1118.

*5.4.9. 5-((Cyclohexylamino)methyl)-6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**8i**).*

Compound **13p** was synthesized from compound **12a** (0.75 mmol, 200 mg), formalin (37%, 1.12 mmol, 91 μL) and cyclohexylamine (1.12 mmol, 128 μL) following the procedure described in *5.3*. Pale yellow solid (61 mg, 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* =8.8 Hz, 1H), 7.15(s, 1H), 7.11 (d, *J* = 8.8 Hz, 2H), 6.79 (s, 1H), 4.74 (s, 2H), 3.86 (s, 3H), 2.56-2.53 (m, 1H), 1.92-1.54 (m, 10H).

The title compound was synthesized from compound **13p** (0.13 mmol, 52 mg) following the typical procedure. Pale yellow solid (30 mg, 60%). mp: 222-225 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 9.2 Hz, 1H), 7.42 (d, *J* = 9.2 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (s, 1H), 4.77 (s, 2H), 3.28 (brs, 1H), 2.26-2.23 (m, 2H), 1.94-1.73 (m, 3H), 1.53-1.26 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD) δ 181.02, 164.16, 161.53, 154.19, 151.17, 128.09, 122.51, 121.42, 121.08, 115.68, 113.71, 104.69, 57.57, 39.54, 29.07, 24.71, 24.15. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{24}O_4N$: 366.1700, found: 366.1708.

*5.5. Synthesis of compound **20***

*5.5.1. 1-(2-Hydroxy-5-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)ethan-1-one (**14**)*

Compound **9** (3 mmol, 456 mg) was dissolved in dichloromethane (15 mL) and stirred at room temperature. Pyridinium *p*-toluene sulfonate (1 mmol, 251 mg) was added followed by the dropwise addition of 3,4-dihydro-2*H*-pyran (3 mmol, 273 μL) in dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was washed with saturated aqueous sodium bicarbonate and extracted with dichloromethane (15 mL × 3). The organic fractions were combined and dried over anhydrous sodium sulfate, evaporated in vacuo, and purified by flash chromatography eluting with *n*-hexanes and ethyl acetate to yield compound **14** (yellow oil, 584 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.89 (s, 1H), 7.41 (s, 1H), 7.24 (s, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 5.31 (s, 1H), 3.93 (t, *J* = 11.0 Hz, 1H), 3.62 (d, *J* = 11.0 Hz, 1H), 2.61 (s, 3H), 1.86–1.55 (m, 6H).

5.5.2.

*(E)-3-(Dimethylamino)-1-(2-hydroxy-5-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)prop-2-en-1-one (**15**).*

The mixture of compound **14** (2.62 mmol, 584 mg) and *N*,*N*-dimethylformamide-dimethylacetal (3.83 mmol, 522 μL) was stirred at 95 °C for 4 h. The reaction mixture was evaporated in vacuo and purified by flash chromatography eluting with n-hexanes and ethyl acetate to yield compound **15** (yellow solid, 382 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 11.5 Hz, 1H), 7.37 (s, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.92-6.81 (m, 1H), 5.72 (d, *J* = 12.0 Hz, 1H), 5.28 (s, 1H), 3.98-3.89 (m, 1H), 3.67-3.55 (m, 1H), 3.18 (s, 3H), 2.97 (s, 3H), 1.86-1.54 (m, 6H).

*5.5.3. 3-Iodo-6-((tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (**16**).*

Compound **15** (1.31 mmol, 382 mg) was dissolved in chloroform (10 mL). Pyridine (1.44 mmol, 114 mg) was added followed by iodine (2.62 mmol, 666 mg). The reaction mixture was stirred at room temperature for 7 h. The mixture was poured into saturated sodium thiosulphate pentahydrate and stirred at room temperature for 30 mins. The mixture was then extracted with dichloromethane (15 mL×3) and the

combined organic layer dried using anhydrous sodium sulphate. The solvent was evaporated in vacuo and the residue was purified by flash chromatography eluted with n-hexanes and ethyl acetate to give compound **16** (white solid, 384 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.82 (d, *J* = 1.6 Hz, 1H), 7.41-7.40 (m, 2H), 5.50 (t, *J* = 3.2 Hz, 1H), 3.90-3.84 (m, 1H), 3.65-3.60 (m, 1H), 1.90-1.53(m, 6H).

*5.5.4. 3-(4-(Benzyloxy)phenyl)-6-((tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (**17**).*

Compound **16** (1.03 mmol, 384 mg) was suspended in dimethoxyethane/water (1:1, 10 mL) and stirred at room temperature. Sodium carbonate (3.09 mmol, 328 mg) was added followed by the 4-benzyloxybenzeneboronic acid (1.24 mmol, 283 mg). 10% Pd/C (0.05 mmol, 55 mg) was added and the reaction mixture was stirred at 45 °C for 4 h under Ar_2. The reaction mixture was filtered through a short pad of celite. The catalyst was washed using water, and then the filter was extracted with dichloromethane (15 mL×3). The organic layer was dried using anhydrous sodium sulphate and the solvent evaporated in vacuo. The residue was purified by flash chromatography eluted using n-hexanes and ethyl acetate to give compound **17** (off-white solid, 253 mg, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99-7.85 (m, 2H), 7.51-7.33 (m, 9H), 7.04 (d, *J* = 7.5 Hz, 2H), 5.51 (s, 1H), 5.11 (s, 2H), 4.01-3.89 (m, 1H), 3.65-3.55 (m, 1H), 1.69-1.54 (m, 6H).

*5.5.5. 3-(4-(Benzyloxy)phenyl)-6-hydroxy-4H-chromen-4-one (**18**).*

p-Toluenesulfonic acid (0.059 mmol, 10 mg) was added to a solution of compound **17** (0.59 mmol, 253 mg) in a 1:1 mixture of methanol and tetrahydrofuran (10 mL). The reaction mixture was stirred at 60 °C for 1 h. The reaction was quenched with triethylamine (0.59 mmol, 82 μL). Compound **18** was synthesized by filtration (pale yellow solid, 130 mg, 64%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.43 (s, 1H), 7.56-7.34 (m, 9H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 2H), 5.16 (s, 2H).

*5.5.6. 3-(4-(Benzyloxy)phenyl)-6-hydroxy-5-(piperidin-1-ylmethyl)-4H-chromen-4-one (**19**).*

To a solution of compound **18** (0.38 mmol, 130 mg) in methanol (15 mL) was added formalin (37%, 0.57 mmol, 46 μL) and piperidine (0.57 mmol, 56 μL). The reaction mixture was stirred for approximately 48 h under argon at 70 °C. The reaction solution was then cooled to room temperature and concentrated in vacuo. Crude residue was purified by column chromatography to give compound **19** (pale yellow solid, 77 mg, 46.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.47-7.34 (m, 8H), 7.14 (d, *J* = 9.2 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 5.16 (s, 2H), 4.60 (s, 2H), 2.51 (brs, 4H), 1.56-1.46 (m, 6H).

*5.5.7. 6-Hydroxy-3-(4-hydroxyphenyl)-5-(piperidin-1-ylmethyl)-4H-chromen-4-one (**20**).*

Compound **19** (0.17 mmol, 77 mg) was dissolved in tetrahydrofuran (10 mL). Ten drops of methanol were added followed by Raney-Ni. The reaction mixture was stirred for 2 h at room temperature under H_2 atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by chromatography to give compound **20** (yellow solid, 23 mg, 37%). mp: 175-177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 9.2 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 2H), 4.55 (s, 2H), 2.48 (brs, 4H), 1.53-1.41 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 178.34, 157.72, 157.23, 152.54, 150.97, 130.95, 124.53, 123.66, 123.32, 123.16, 119.21, 119.01, 115.52, 57.85, 53.75, 25.89, 23.85. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{22}O_4N$: 352.1543, found: 352.1550.

*5.6. Synthesis of compounds **29a–e** and **32a–c***

*5.6.1. Synthesis of compounds **26a,b***

Compounds **25a,b** were prepared from diethyl malonate (**21**) and benzoyl chlorides **22a,b** following the procedure described in the literature [19,20]. Compounds **25a,b**

(1 equiv) and polyphosphoric acid (2 equiv) were heated at 100 °C for 4 h, and then the mixture was poured into ice water and extracted with dichloromethane (15 mL × 3). The organic layers were combined, washed with saturated aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and evaporated under vacuum. Crude residue was purified by chromatography to give compounds **26a,b**.

*5.6.1.1. Ethyl 6-methoxy-4-oxo-2-phenyl-4H-chromene-3-carboxylate (**26a**).*

Compound **26a** was synthesized from compound **25a** (10 mmol, 3.7 g) and polyphosphoric acid (100 mmol, 34 g) following the typical procedure. Yellow solid (0.97 g, 30%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.0 Hz, 2H), 7.62 (d, *J* = 2.5 Hz, 1H), 7.55-7.45 (m, 4H), 7.30 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.28 (q, *J* = 7.0 Hz, 2H), 3.91 (s, 3H), 1.17 (t, *J* = 7.0 Hz, 3H).

*5.6.1.2. Ethyl 6-methoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**26b**).*

Compound **26b** was synthesized from compound **25b** (10 mmol, 4 g) and polyphosphoric acid (100 mmol, 34 g) following the typical procedure. Yellow solid (1.17 g, 33%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, *J* = 8.5 Hz, 3H), 7.47-7.43 (m, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 4.22 (q, *J* = 7.0 Hz, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 1.15 (t, *J* = 7.0 Hz, 3H).

*5.6.2. Synthesis of compounds **28a,b***

Boron tribromide (3 or 6 equiv) was added to a solution of **26a,b** (1 equiv) in dichloromethane (10 mL) at -78 °C under argon, and then the mixture was allowed to warm to room temperature and was stirred for 5 h. The reaction was quenched with methanol and evaporated under reduced pressure. Compounds **27a,b** were obtained by chromatography. Concentrated hydrochloric acid (3 equiv) was added to a solution of **27a,b** (1 equiv) in ethanol (10 mL). The reaction mixture was stirred for approximately 12 h at 80 °C and cooled to room temperature. Water was added and the mixture was extracted with dichloromethane (15 mL × 3). The combined organic

layers were washed with saturated aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and evaporated under vacuum to give compounds **28a,b**.

*5.6.2.1. Ethyl 6-hydroxy-4-oxo-2-phenyl-4H-chromene-3-carboxylate (**28a**).*

Compound **28a** was synthesized from compound **26a** (3 mmol, 972 mg) following procedure. Off-white solid (0.82 g, 88%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.65-7.59 (m, 4H), 7.35 (s, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 4.16 (q, *J* = 7.0 Hz, 2H), 1.08 (t, *J* = 7.0 Hz, 3H).

*5.6.2.2. Ethyl 6-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**28b**).*

Compound **28b** was synthesized from compound **26b** (3.3 mmol, 1168 mg) following procedure. Brown solid (1.02 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.12 (s, 1H), 7.62-7.59 (m, 3H), 7.33-7.27 (m, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 1.14 (t, *J* = 7.2 Hz, 3H).

*5.6.3. Synthesis of compounds **29a–e***

Formalin (37%, 1.5 equiv) and an amine derivative (1.5 equiv) were added to a solution of **28a,b** in ethanol (15 mL). The reaction mixture was stirred for 12–48 h under argon at 80 °C. The reaction solution was cooled to room temperature and concentrated in vacuo. Crude residue was purified by chromatography to give compounds **29a–e**.

*5.6.3.1. Ethyl 6-hydroxy-4-oxo-2-phenyl-5-(piperidin-1-ylmethyl)-4H-chromene-3-carboxylate (**29a**).*

The title compound was synthesized from compound **28a** (0.4 mmol, 123 mg), formalin (37%, 0.6 mmol, 48 μL) and piperidine (0.6 mmol, 59 μL) following the typical procedure. Pale yellow solid (66 mg, 41%). mp: 115-117 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, *J* = 5.5 Hz, 2H), 7.62-7.55 (m, 4H), 7.20 (d, *J* = 8.5 Hz, 1H), 4.59 (s, 2H), 4.17 (q, *J* = 6.5 Hz, 2H), 2.55 (s, 4H), 1.57(s, 4H), 1.47(s, 2H), 1.11

(t, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.77, 165.29, 160.54, 157.71, 150.52, 132.16, 131.61, 129.46, 128.14, 124.25, 121.32, 119.20, 119.00, 118.47, 61.81, 57.23, 53.55, 25.72, 23.66, 14.16. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{26}O_5N$: 408.1806, found: 408.1806.

*5.6.3.2. Ethyl 6-hydroxy-4-oxo-2-phenyl-5-(pyrrolidin-1-ylmethyl)-4H-chromene-3-carboxylate (**29b**).*

The title compound was synthesized from compound **28a** (0.48 mmol, 148 mg), formalin (37%, 0.72 mmol, 58 μL) and pyrrolidine (0.72 mmol, 59 μL) following the typical procedure. Pale yellow solid (71 mg, 38%). mp: 112-114 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, *J* = 7.2 Hz, 2H), 7.63-7.55 (m, 4H), 7.21 (d, *J* = 8.8 Hz, 1H), 4.73 (s, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.69 (s, 4H), 1.81 (s, 4H), 1.10 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.72, 165.29, 160.60, 157.70, 150.38, 132.15, 131.64, 129.45, 128.14, 124.21, 120.98, 119.68, 119.26, 118.47, 61.81, 53.99, 53.40, 23.72, 14.15. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{24}O_5N$: 394.1649, found: 394.1661.

*5.6.3.3. Ethyl 6-hydroxy-2-(4-hydroxyphenyl)-4-oxo-5-(pyrrolidin-1-ylmethyl)-4H-chromene-3-carboxylate (**29c**).*

The title compound was synthesized from compound **28b** (0.31 mmol, 100 mg), formalin (37%, 0.46 mmol, 37 μL) and pyrrolidine (0.46 mmol, 38 μL) following the typical procedure. Pale yellow solid (44 mg, 35%). mp: 140-142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.59 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 9.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 5.76 (s, 1H), 4.72 (s, 2H), 4.20 (q, *J* = 7.0 Hz, 2H), 2.67 (s, 4H), 1.81 (s, 4H), 1.16 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.79, 165.82, 161.28, 160.50, 157.53, 150.18, 130.03, 123.96, 121.82, 120.84, 119.55, 119.14, 117.05, 116.30, 61.76, 53.92, 53.40, 23.71, 14.25. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{24}O_6N$: 410.1598, found: 410.1591.

5.6.3.4. Ethyl 5-(azetidin-1-ylmethyl)-6-hydroxy-4-oxo-2-phenyl-4H-chromene-3-

*carboxylate (**29d**).*

The title compound was synthesized from compound **28a** (0.47 mmol, 145 mg), formalin (37%, 0.6 mmol, 57 μL) and azetidine (0.7 mmol, 47 μL) following the typical procedure. Pale yellow solid (60 mg, 34%). mp: 125-127 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73-7.71 (m, 2H), 7.65-7.55 (m, 4H), 7.23 (d, *J* = 9.2 Hz, 1H), 4.65 (s, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.40 (t, *J* = 7.2 Hz, 4H), 2.12 (t, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.66, 165.29, 160.67, 157.34, 150.41, 132.18, 131.59, 129.46, 128.14, 124.37, 121.26, 119.51, 118.56，118.44, 61.85, 56.44, 54.66, 17.18, 14.15. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{22}O_5N$: 380.1492, found: 380.1475.

*5.6.3.5. Ethyl 5-(azetidin-1-ylmethyl)-6-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**29e**).*

The title compound was synthesized from compound **28b** (0.31 mmol, 100 mg), formalin (37%, 0.46 mmol, 37 μL) and azetidine (0.46 mmol, 31 μL) following the typical procedure. Off-white solid (68 mg, 56%). mp: >250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.59 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 9.0 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 2H), 4.65 (s, 2H), 4.56 (s, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 3.36 (t, *J* = 7.0 Hz, 4H), 2.11 (t, *J* = 7.0 Hz, 2H), 1.17 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.71, 165.81, 161.25, 160.50, 157.15, 150.25, 130.03, 124.07, 121.82, 121.09, 119.27, 118.73, 117.06, 116.30, 61.77, 56.67, 54.64, 17.20, 14.26. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{22}O_6N$: 396.1442, found: 396.1443.

*5.7. Synthesis of compounds **32a–c***

*5.7.1. 6-Hydroxy-N-methyl-4-oxo-2-phenyl-4H-chromene-3-carboxamide (**31**)*

Oxalyl chloride (1.62 mmol, 138 μL) and *N*,*N*-dimethylformamide (2 drops) were added to a solution of acid **27a** (1.24 mmol, 351 mg) in dichloromethane (10 mL) at 0 °C and stirred at room temperature for 4 h. The reaction mixture was evaporated under reduced pressure to give compound **30**. Compound **30** was dissolved in THF

(10 mL), a solution of methylamine (2.48 mmol, 1.24 mL, 2 M in THF) was added, and the mixture was stirred for 4 h at room temperature. After the reaction was complete, the solvent was evaporated. Water was added and the mixture was extracted with dichloromethane, and the organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by chromatography (CH_2Cl_2:CH_3OH = 98:2 v/v) to give compound **31** (brown-yellow solid, 186 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.25 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.63 – 7.54 (m, 4H), 7.34 (s, 1H), 7.28 (d, *J* = 9.2 Hz, 1H), 2.61 (d, *J* = 4.0 Hz, 3H).

*5.7.2. 6-Hydroxy-N-methyl-4-oxo-2-phenyl-5-(piperidin-1-ylmethyl)-4H-chromene-3-carboxamide (**32a**).*

The title compound was synthesized from compound **31** (0.13 mmol, 38 mg), formalin (37%, 0.1 9 mmol, 19 μL) and piperidine (0.19 mmol, 16 μL) following the typical procedure. Pale yellow solid (19 mg, 38%). mp: 110-113 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, *J* = 4.8 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.58-7.52 (m, 4H), 7.21 (d, *J* = 8.8 Hz, 1H), 4.60 (s, 2H), 2.61 (d, *J* = 4.4 Hz, 7H), 1.60-1.48 (m, 6H). ^{13}CNMR (100 MHz, DMSO-d_6) δ 177.68, 164.78, 159.05, 157.14, 150.45, 132.14, 131.71, 129.19, 128.22, 123.95, 121.73, 121.39, 119.34, 118.34, 56.72, 53.54, 26.35, 25.42, 23.47. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{25}O_4N_2$:393.1809, found: 393.1800.

*5.7.3. 6-Hydroxy-N-methyl-4-oxo-2-phenyl-5-(pyrrolidin-1-ylmethyl)-4H-chromene-3-carboxamide (**32b**).*

The title compound was synthesized from compound **31** (0.32 mmol, 93 mg), formalin (37%, 0.47 mmol, 38 μL) and pyrrolidine (0.47 mmol, 39 μL) following the typical procedure. Yellow solid (68 mg, 57%). mp: 79-82 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, *J* = 4.8 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.58-7.53 (m, 4H), 7.18 (d, *J* = 8.8 Hz, 1H), 4.73 (s, 2H), 2.66 (s, 4H), 2.61 (d, *J* = 4.8 Hz, 3H), 1.81 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.61, 164.86, 158.98, 157.39, 150.30,

132.19, 131.65, 129.17, 128.22, 123.91, 121.38, 121.26, 119.70, 119.01, 54.11, 53.40, 26.35, 23.71. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{23}O_4N_2$: 379.1652, found: 379.1667.

*5.7.4. 5-(Azetidin-1-ylmethyl)-6-hydroxy-N-methyl-4-oxo-2-phenyl-4H-chromene-3-carboxamide (**32c**).*

The title compound was synthesized from compound **31** (0.32 mmol, 93 mg), formalin (37%, 0.47 mmol, 38 μL) and azetidine (0.47 mmol, 32 μL) following the typical procedure. Yellow solid (48 mg, 55%). mp: 147-150 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 4.8 Hz, 1H), 7.81 (dd, J = 8.4, 2.0 Hz, 2H), 7.59-7.52 (m, 4H), 7.22 (d, J = 9.2 Hz, 1H), 4.67 (s, 2H), 3.43 (t, J = 7.2 Hz, 4H), 2.62 (d, J = 4.4 Hz, 3H), 2.22-2.06 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.55, 164.81, 159.06, 156.89, 150.36, 132.16, 131.68, 129.18, 128.23, 124.02, 121.57, 121.38, 119.34, 118.49, 56.47, 54.67, 26.36, 17.21. HR-MS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{21}O_4N_2$: 365.1496, found: 365.1506.

5.8. Biological assays (Supporting information)

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/

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Highlights

• Novel 4*H*-chromen-4-one derivatives were identified as anti-TB agents.

• Compound **8d** exhibited good activity against DS-TB and MDR-TB.

• Compound **8d** exhibited good druggability profiles.

• Compound **8d** was an excellent lead for further optimization to develop anti-TB drugs.

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: