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Diversity-oriented synthesis of amide derivatives of tricyclic thieno[2,3-*d*]pyrimidin-4(3*H*)-ones and evaluation of their influence on melanin synthesis in murine B16 cells

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Abstract: A diversity-oriented synthesis of amide-containing thieno[2,3-*d*]pyrimidin-4(3*H*)-ones is reported. All compounds were tested for their influence on melanin synthesis in murine B16 cells. The azepine fragment in thieno[2,3-*d*]pyrimidin-4(3*H*)-one skeleton significantly increases the melanin content.

Keywords: melanin synthesis; murine B16 cells; thieno[2,3-*d*]pyrimidin-4(3*H*)-one; vitiligo.

Introduction

Vitiligo is a disease characterized by loss of pigment cells in the epidermis [1]. It can involve any part of the body where melanocytes reside and cause both functional and physiological abnormalities in the affected skin. So far, several theories have been generated to explain the pathogenesis of the vitiligo disease [2]. It is generally believed that the disease results from the destruction of melanocytes and obstruction of the melanin synthesis [3].

Presently, there are several drugs used for the treatment of vitiligo including topical corticosteroids, calcineurin inhibitors, vitamin D_3 analogs and psoralens [4]. Several psoralens (furocoumarins) have been clinically applied, including 8-methoxypsoralen (8-MOP, Figure 1) [5, 6], 5-methoxypsoralen [7] and 4,5,8-trimethylpsoralen [8, 9]. These compounds show strong photosensitivity, which may be used for the treatment of vitiligo with subsequent exposure to long-wave ultraviolet (UV) radiation. Nowadays, this therapy is still the most successful approach for the treatment of vitiligo, although there are some undesired side effects such as genetic mutation and risk of skin cancer [10, 11]. In order to discover new active drug candidates, our group has synthesized dozens of psoralen derivatives. *In vitro* evaluation has indicated that some of them possess good stimulation effects on tyrosinase and melanin synthesis in murine B16 cells [12, 13].

Thieno[2,3-*d*]pyrimidin-4(3*H*)-one is a common building block for drugs with diverse pharmaceutical activities [14–16]. It has been widely used for the preparation of new antibacterial [17, 18] and antitumor agents [19], among other drugs. As can be seen from Figure 1, the core of thieno[2,3-*d*]pyrimidin-4(3*H*)-one is similar to that of psoralen, and we envisioned that derivatives of thieno[2,3-*d*]pyrimidin-4(3*H*)-one might function similarly to psoralen derivatives. Recently, 18 new sulfonamides derived from 6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidin-4-one have been synthesized by our group. Several compounds exhibited more than 1.5-fold potency as compared to 8-MOP [5]. In the present study, inspired by these positive results, we synthesized 51 novel amide derivatives of tricyclic thieno[2,3-*d*]pyrimidines and investigated their activities on melanin synthesis in murine B16 cells.

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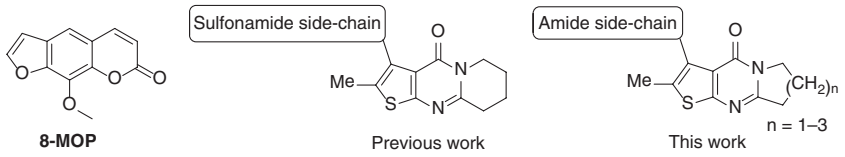


Figure 1 Structures of 8-methoxypsolaren (8-MOP) and related derivatives of thieno[2,3-*d*]pyrimidin-4(3*H*)-one [5].

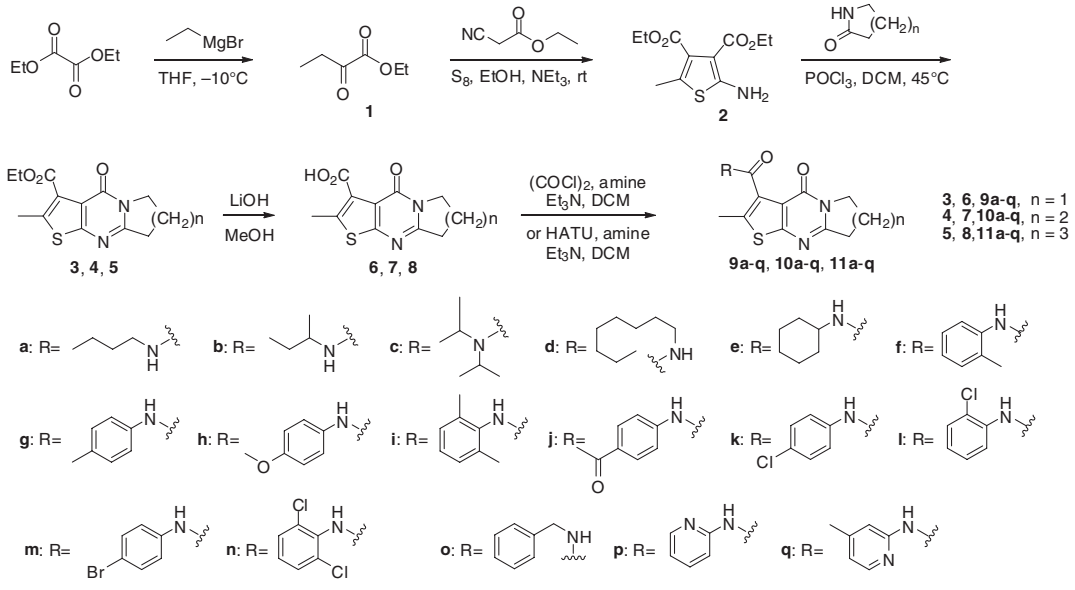
Results and discussion

Synthesis

The synthetic route of the target compounds (Scheme 1) follows our previous procedure [5]. An intermediate product **1** was prepared from diethyl oxalate via the Grignard reaction. Application of the Gewald reaction to compound **1**, cyanoacetate and sulfur in the presence of triethylamine at room temperature produced compound **2** in high yield. Then, ethyl thieno[2,3-*d*]pyrimidine carboxylates **3–5** were prepared through the condensation of compound **2** and cyclic lactams in the presence of phosphorus oxychloride in dichloromethane at 45°C. Hydrolysis of esters **3–5** was completed in a mixture of water and methanol in the presence of lithium hydroxide to yield the acids **6–8**. In order to obtain the amidation products in good yields, the optimization reactions with acid **6** under different conditions using a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and hydroxybenzotriazole (EDCI and HOBt), (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxide hexafluorophosphate (HATU) and oxalyl chloride as activating reagents were conducted. *n*-Butylamine and 1-(4-aminophenyl)ethan-1-one were selected to represent aliphatic and aromatic amines, respectively. Using the coupling reagents EDCI and HOBt, the yields were only moderate for both aliphatic amine (53%) and aromatic amine (65%). HATU provided a good yield for the reaction of aliphatic amine (88%), slightly better than for aromatic amine (74%). The conversion of acid **6** to carboxylic chloride and then condensation with amine provided excellent yields with the aromatic amine (90%), much better than for the aliphatic *n*-butylamine. Therefore, it was decided to synthesize the target compounds **9a–e**, **10a–e**, **11a–e** bearing the aliphatic amino moiety in the presence of HATU and to prepare compounds **9f–q**, **10f–q**, **11f–q** with aromatic amine moiety from carboxylic acid chloride.

Melanin synthesis evaluation

All the tricyclic thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives **9a–q**, **10a–q**, **11a–q** were tested for their effect on melanin synthesis in murine B16 cells, following a previously published method [20]. As shown in Table 1, the reference drug 8-MOP enhances the melanin synthesis by 126.1 ± 3.2% at 50 μM as compared to the blank control.



Scheme 1 Synthetic route to compounds **9a–q**, **10a–q** and **11a–q**.

Table 1 Stimulation of melanin content of B16 cells by compounds **9a–q**, **10a–q**, **11a–q**.

Compound	Relative melain contents (%)	Compound	Relative melain contents (%)	Compound	Relative melain contents (%)
9a	87.2 ± 2.5	**10a**	109.1 ± 9.9	**11a**	97.1 ± 3.2
9b	125.6 ± 3.4	**10b**	105.9 ± 1.4	**11b**	155.8 ± 5.6
9c	126.4 ± 5.5	**10c**	133.2 ± 1.8	**11c**	161.7 ± 7.1
9d	151.4 ± 6.4	**10d**	174.5 ± 4.7	**11d**	111.5 ± 9.8
9e	125.6 ± 3.4	**10e**	159.6 ± 5.1	**11e**	49.1 ± 7.6
9f	85.5 ± 6.9	**10f**	151.9 ± 5.2	**11f**	143.9 ± 6.3
9g	88.5 ± 7.8	**10g**	103.0 ± 6.8	**11g**	173.4 ± 10.8
9h	103.5 ± 5.2	**10h**	227.6 ± 4.3	**11h**	82.9 ± 13.1
9i	147.1 ± 2.6	**10i**	112.5 ± 7.8	**11i**	116.6 ± 3.7
9j	122.5 ± 7.1	**10j**	126.4 ± 9.5	**11j**	92.5 ± 10.9
9k	96.1 ± 8.4	**10k**	187.9 ± 8.6	**11k**	105.3 ± 6.1
9l	116.6 ± 6.8	**10l**	155.8 ± 7.9	**11l**	147.0 ± 4.3
9m	114.7 ± 4.9	**10m**	201.2 ± 6.7	**11m**	136.7 ± 8.2
9n	113.1 ± 5.6	**10n**	154.4 ± 5.6	**11n**	234.5 ± 5.9
9o	116.9 ± 7.1	**10o**	130.7 ± 6.2	**11o**	94.5 ± 10.7
9p	114.6 ± 7.6	**10p**	154.4 ±8 .6	**11p**	150.4 ± 4.6
9q	75.1 ± 4.6	**10q**	95.4 ± 3.9	**11q**	40.6 ± 11.4
8-MOP			126.1 ± 3.2		

The data were recorded as the mean ± SD% of three experiments in duplicate and compared to the blank control.

The effectiveness of the synthetic compounds on melanin synthesis varies from 40.6 ± 11.4 to 234.5 ± 5.9%. It can be noticed that the five-membered ring compounds (**9a–q**, n = 1) showed decreased melanin synthesis, no matter what kind of substituent group is present on the amide moiety. Among these 17 compounds, only compounds **9d** and **9i** showed higher values than 8-MOP. Several compounds fused with a seven-membered ring (**11**, n = 3) possess better activities to promote melanin content. Some six-membered derivatives (**10**, n = 2) show high potency. Most of them (11 out of 17) increase the synthesis of melanin. It can be concluded that the size of fused rings of the synthetic compounds dramatically affects their ability to influence melanin synthesis.

Substituents on the amide moiety also contribute to the biological effectiveness. For example, compounds with *n*-octylamino group **9d**, **10d**, **11d** exhibit an increased potency in comparison to the derivatives substituted with an *n*-butylamino group **9a**, **10a**, **11a**. Compound **11n** is the lead candidate showing the highest potency on melanin synthesis in murine B16 cells, with a value of 234.5 ± 5.9%, which is 1.86-fold greater compared to that of 8-MOP. Another hit compound is **10h** which contains electron-donating substituent (methoxy group) attached to position 4 of the aromatic ring and increases the melanin content by 227.6 ± 4.3%. Interestingly, activities of compounds with a 2,6-dichlorophenyl fragment in amide linker increase with the increase in size of the saturated ring **9n** < **10n** < **11n**.

Conclusion

New amide-substituted tricyclic thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives were synthesized and evaluated for their effect on melanin synthesis in murine B16 cells. Several synthetic compounds are more potent than the standard drug 8-methoxypsolaren.

Experimental

Reagents and solvents were purchased from Sigma and used without purification. Thin-layer chromatography (TLC) was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254); spots were visualized by illumination with UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh). Melting points were determined on a Buchi B-540 apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR), (400 MHz) and carbon-13 nuclear magnetic resonance (^{13}C NMR) (100 MHz) spectra were recorded on a Varian NMR spectrometer in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were recorded on an AB SCIEX QSTAR Elite quadrupole time-of-flight mass spectrometer.

General procedure for the synthesis of compounds 9a–q, 10a–q and 11a–q

Method A To a solution of 2-methyl-4-oxo-thieno[2,3-*d*]pyrimidine-3-carboxylic acid (**6–8**, 1.0 mmol) and HATU (1.2 mmol) in

dichloromethane (20 mL) was added *N*,*N*-diisopropylethylamine (DIPEA, 0.27 mL, 1.5 mmol) and an aliphatic amine (see Scheme 1, 1.5 mmol) dropwise at 0–5°C. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was extracted with dichloromethane (2×30 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to produce the pure compounds **9a–e**, **10a–e**, **11a–e**.

Method B A solution of 2-methyl-4-oxo-thieno[2,3-*d*]pyrimidine-3-carboxylic acid (**6–8**, 1.0 mmol) in dichloromethane (10 mL) was treated with oxalyl chloride (3 mmol) and the mixture was stirred at room temperature for 3 h. The solvent and the excess of oxalyl chloride were removed under reduced pressure and the residue was treated with an aromatic amine (see Scheme 1, 1.1 mmol) and triethylamine (1.2 mmol). The mixture was stirred at room temperature for 8 h, then washed with water and concentrated under reduced pressure. The residue was purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate to give the pure compounds **9f–q**, **10f–q**, **11f–q**.

***N*-Butyl-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9a)** Yield 81%; light yellow solid; mp 119–121°C; ^{1}H NMR: δ 10.40 (d, *J*=3.8 Hz, 1H), 4.23 (t, *J*=7 Hz, 2H), 3.41 (t, *J*=7 Hz, 2H), 3.20 (t, *J*=8 Hz, 2H), 2.87 (s, 3H), 2.38–2.30 (m, 2H), 1.69–1.59 (m, 2H), 1.49–1.39 (m, 2H), 0.96 (t, *J*=7 Hz, 3H); ^{13}C NMR: δ 163.5, 163.3, 159.2, 158.6, 145.8, 126.3, 118.6, 47.4, 39.5, 32.0, 31.5, 20.4, 19.6, 17.5, 13.9. HRMS (ESI). Calcd for $C_{15}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 305.1193. Found: *m/z* 305.1198.

***N*-(*sec*-Butyl)-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9b)** Yield 89%; light yellow solid; mp 113–115°C; ^{1}H NMR: δ 10.21 (d, *J*=4.3 Hz, 1H), 4.23 (t, *J*=7 Hz, 2H), 4.09–4.01 (m, 1H), 3.20 (t, *J*=8 Hz, 2H), 2.86 (s, 3H), 2.38–2.30 (m, 2H), 1.72–1.53 (m, 2H), 1.25 (d, *J*=7 Hz, 3H), 0.98 (t, *J*=7 Hz, 3H); ^{13}C NMR: δ 163.4, 162.6, 159.2, 158.5, 145.5, 126.7, 118.6, 46.8, 38.7, 32.0, 29.5, 20.1, 19.6, 17.5, 10.6. HRMS (ESI). Calcd for $C_{15}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 305.1192. Found: *m/z* 305.1198.

***N*,*N*-Diisopropyl-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9c)** Yield 82%; white solid; mp 207–209°C; ^{1}H NMR: δ 4.16 (t, *J*=7 Hz, 2H), 3.79–3.72 (m, 1H), 3.57–3.50 (m, 1H), 3.16 (t, *J*=8 Hz, 2H), 2.44 (s, 3H), 2.28 (m, 2H), 1.67 (d, *J*=7 Hz, 3H), 1.58 (d, *J*=7 Hz, 3H), 1.14 (t, *J*=7 Hz, 6H); ^{13}C NMR: δ 164.8, 163.6, 159.9, 156.2, 131.5, 130.8, 120.0, 51.2, 46.5, 46.0, 32.2, 21.3, 20.8, 20.2, 19.9, 19.7, 13.7. HRMS (ESI). Calcd for $C_{17}H_{23}N_3O_2S$ [M–H]$^-$: *m/z* 333.1507. Found: *m/z* 333.1511.

2-Methyl-*N*-octyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9d) Yield 76%; white solid; mp 102–104°C; ^{1}H NMR: δ 10.40 (d, *J*=3.5 Hz, 1H), 4.23 (t, *J*=7 Hz, 2H), 3.43–3.36 (m, 2H), 3.20 (t, *J*=8 Hz, 2H), 2.87 (s, 3H), 2.38–2.30 (m, 2H), 1.68–1.61 (m, 2H), 1.43–1.23 (m, 10H), 0.87 (t, *J*=7 Hz, 3H); ^{13}C NMR: δ 163.5, 163.2, 159.2, 158.6, 145.8, 126.4, 118.6, 47.3, 39.7, 32.0, 31.8, 29.4, 29.3, 29.2, 27.2, 22.7, 19.6, 17.5, 14.1. HRMS (ESI). Calcd for $C_{19}H_{27}N_3O_2S$ [M–H]$^-$: *m/z* 361.1831. Found: *m/z* 361.1824.

***N*-Cyclohexyl-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9e)** Yield 87%; light yellow solid; mp 186–188°C; ^{1}H NMR: δ 10.27 (d, *J*=7 Hz, 1H), 4.23 (t, *J*=7 Hz, 2H), 3.98–3.87 (m, 1H), 3.20 (t, *J*=8 Hz, 2H), 2.86 (s, 3H), 2.38–2.30 (m, 2H), 2.06–1.99 (m, 2H), 1.82–1.73 (m, 2H), 1.58–1.17 (m, 6H); ^{13}C NMR: δ 163.4, 162.3, 159.2, 158.5, 145.6, 126.6, 118.7, 48.5, 47.4, 32.9, 32.0, 25.8, 25.0, 19.6, 17.5. HRMS (ESI). Calcd for $C_{17}H_{21}N_3O_2S$ [M–H]$^-$ *m/z* 331.1348. Found: *m/z* 331.1354.

2-Methyl-4-oxo-*N*-(o-tolyl)-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9f) Yield 87%; white solid; mp 213–215°C; ^{1}H NMR: δ 12.45 (s, 1H), 7.68 (d, *J*=8 Hz, 1H), 7.25–7.18 (m, 2H), 7.12–7.05 (m, 1H), 4.15 (t, *J*=7 Hz, 2H), 3.10 (t, *J*=7 Hz, 2H), 2.89 (s, 3H), 2.42–2.35 (m, 2H), 2.35 (s, 3H); ^{13}C NMR: δ 162.7, 161.7, 159.0, 158.4, 147.1, 135.9, 132.4, 129.4, 126.1, 120.5, 118.7, 45.2, 32.8, 23.6, 19.5, 17.7. HRMS (ESI). Calcd for $C_{18}H_{17}N_3O_2S$ [M–H]$^-$: *m/z* 339.1049. Found: *m/z* 339.1041.

2-Methyl-4-oxo-*N*-(*o*-tolyl)-4,6,7,8-tetrahydropyrrolo[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (9g) Yield 79%; white solid; mp 257–259°C; ^{1}H NMR: δ 12.77 (s, 1H), 7.67 (d, *J*=8 Hz, 2H), 7.14 (d, *J*=8 Hz, 2H), 4.25 (t, *J*=7 Hz, 2H), 3.20 (t, *J*=8 Hz, 2H), 2.93 (s, 3H), 2.39–2.30 (m, 2H), 2.33 (s, 3H); ^{13}C NMR: δ 163.4, 161.0, 159.3, 159.0, 147.5, 136.6, 133.1, 129.3, 126.4, 120.3, 118.2, 47.5, 31.9, 20.9, 19.5, 18.0. HRMS (ESI). Calcd for $C_{18}H_{17}N_3O_2S$ [M–H]$^-$: *m/z* 339.1048. Found: *m/z* 339.1041.

***N*-(4-Methoxyphenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydro-pyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9h)** Yield 76%; light yellow solid; mp 201–203°C; ^{1}H NMR: δ 12.70 (s, 1H), 7.69 (d, *J*=9 Hz, 2H), 6.88 (d, *J*=9 Hz, 2H), 4.24 (t, *J*=7 Hz, 2H), 3.81 (s, 3H), 3.21 (t, *J*=8 Hz, 2H), 2.92 (s, 3H), 2.39–2.28 (m, 2H); ^{13}C NMR: δ 163.3, 160.9, 159.4, 158.9, 156.0, 147.4, 132.4, 126.3, 121.8, 118.3, 114.0, 55.5, 47.5, 31.9, 19.5, 17.9. HRMS (ESI). Calcd for $C_{18}H_{17}N_3O_3S$ [M–H]$^-$: *m/z* 355.0999. Found: *m/z* 355.0991.

***N*-(2,6-Dimethylphenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydro-pyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9i)** Yield 86%; light yellow solid; mp 215–217°C; ^{1}H NMR: δ 12.02 (s, 1H), 7.14–7.07 (m, 3H), 4.24 (t, *J*=7 Hz, 2H), 3.22 (t, *J*=8.0 Hz, 2H), 2.91 (s, 3H), 2.39–2.32 (m, 2H), 2.31 (s, 6H); ^{13}C NMR: δ 163.5, 161.5, 159.3, 158.8, 147.5, 135.3, 135.1, 127.9, 126.5, 125.8, 118.6, 47.4, 32.0, 19.6, 18.7, 17.8. HRMS (ESI). Calcd for $C_{19}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 353.1191. Found: *m/z* 353.1198.

***N*-(4-Acetylphenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9j)** Yield 89%; light yellow solid; mp 189–191°C; ^{1}H NMR: δ 13.32 (s, 1H), 7.94 (d, *J*=9 Hz, 2H), 7.88 (d, *J*=9 Hz, 2H), 4.26 (t, *J*=7 Hz, 2H), 3.22 (t, *J*=8 Hz, 2H), 2.94 (s, 3H), 2.58 (s, 3H), 2.41–2.32 (m, 2H); ^{13}C NMR: δ 197.0, 163.5, 161.3, 159.5, 159.0, 148.9, 143.9, 132.2, 129.5, 125.8, 119.4, 118.0, 47.6, 31.9, 26.4, 19.5, 18.1. HRMS (ESI). Calcd for $C_{19}H_{17}N_3O_3S$ [M–H]$^-$: *m/z* 367.0984. Found: *m/z* 367.0991.

***N*-(4-Chlorophenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9k)** Yield 82%; white solid; mp 252–254°C; ^{1}H NMR: δ 13.05 (s, 1H), 7.79–7.71 (m, 2H), 7.32–7.27 (m, 2H), 4.26 (t, *J*=7 Hz, 2H), 3.23 (t, *J*=8 Hz, 2H), 2.93 (s, 3H), 2.41–2.33 (m, 2H); ^{13}C NMR: δ 163.6, 161.1, 159.4, 159.0, 148.3, 137.9, 128.7, 128.4, 126.0, 121.4, 118.1, 47.5, 31.9, 19.6, 18.0. HRMS (ESI). Calcd for $C_{17}H_{14}ClN_3O_2S$ [M–H]$^-$: *m/z* 359.0486. Found: *m/z* 359.0495.

***N*-(2-Chlorophenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydro-pyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9l)** Yield 84%; light yellow solid; mp 220–222°C; ^{1}H NMR: δ 12.03

(s, 1H), 8.11 (dd, *J* = 8 Hz, 0.9 Hz, 1H), 7.41 (dd, *J* = 8 Hz, 1.3 Hz, 1H), 7.32–7.27 (m, 1H), 7.09 (td, *J* = 8 Hz, 1.5 Hz, 1H), 4.26 (t, *J* = 7 Hz, 2H), 3.22 (t, *J* = 8 Hz, 2H), 2.88 (s, 3H), 2.39–2.31 (m, 2H); ^{13}C NMR: δ 163.3, 161.7, 159.6, 158.3, 147.4, 135.4, 129.5, 127.0, 126.7, 126.0, 125.5, 125.4, 118.5, 47.4, 32.0, 19.6, 17.7. HRMS (ESI). Calcd for $C_{17}H_{14}ClN_3O_2S$ [M–H]$^-$: *m/z* 359.0483. Found: *m/z* 359.0495.

***N*-(4-Bromophenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9m)** Yield 90%; light yellow solid; mp 224–226°C; ^{1}H NMR: δ 13.06 (s, 1H), 7.71 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8 Hz, 2H), 4.27 (t, *J* = 7 Hz, 2H), 3.23 (t, *J* = 8 Hz, 2H), 2.94 (s, 3H), 2.42–2.32 (m, 2H); ^{13}C NMR: δ 163.6, 161.1, 159.4, 159.1, 148.4, 138.4, 131.7, 126.0, 121.8, 118.1, 116.1, 47.5, 31.9, 19.6, 18.0. HRMS (ESI). Calcd for $C_{17}H_{14}BrN_3O_2S$ [M–H]$^-$: *m/z* 402.9996. Found: *m/z* 402.9990.

***N*-(2,6-Dichlorophenyl)-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9n)** Yield 88%; white solid; mp 267–269°C; ^{1}H NMR: δ 12.27 (s, 1H), 8.09 (d, *J* = 8 Hz, 1H), 7.42 (d, *J* = 2.4 Hz, 1H), 7.29–7.22 (m, 2H), 4.24 (t, *J* = 7 Hz, 2H), 3.23 (t, *J* = 8 Hz, 2H), 2.89 (s, 3H), 2.40–2.31 (m, 2H); ^{13}C NMR: δ 163.4, 161.7, 159.65, 158.5, 148.1, 134.3, 129.9, 129.2, 127.3, 127.2, 126.1, 125.6, 118.4, 47.4, 32.0, 19.6, 17.8. HRMS (ESI). Calcd for $C_{17}H_{13}Cl_2N_3O_2S$ [M–H]$^-$: *m/z* 393.0101. Found: *m/z* 393.0106.

***N*-Benzyl-2-methyl-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9o)** Yield 73%; light yellow solid; mp 142–144°C; ^{1}H NMR: δ 10.96 (d, *J* = 1.1 Hz, 1H), 7.40 (d, *J* = 7 Hz, 2H), 7.32 (t, *J* = 7 Hz, 2H), 7.23 (t, *J* = 7 Hz, 1H), 4.63 (d, *J* = 4 Hz, 2H), 4.19 (t, *J* = 7 Hz, 2H), 3.18 (t, *J* = 8 Hz, 2H), 2.88 (s, 3H), 2.36–2.26 (m, 2H); ^{13}C NMR: δ 163.5, 163.3, 159.3, 158.5, 146.4, 139.0, 128.5, 127.7, 126.9, 126.0, 118.6, 47.4, 43.4, 32.0, 19.6, 17.6. HRMS (ESI). Calcd for $C_{18}H_{17}N_3O_2S$ [M–H]$^-$: *m/z* 339.1035. Found: *m/z* 339.1041.

2-Methyl-4-oxo-*N*-(pyridin-2-yl)-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9p) Yield 76%; light yellow solid; mp 218–220°C; ^{1}H NMR: δ 13.40 (s, 1H), 8.45 (dd, *J* = 5 Hz, 1 Hz, 1H), 8.40 (d, *J* = 8 Hz, 1H), 7.77–7.70 (m, 1H), 7.06–7.01 (m, 1H), 4.33 (t, *J* = 7 Hz, 2H), 3.22 (t, *J* = 8 Hz, 2H), 2.93 (s, 3H), 2.40–2.31 (m, 2H); ^{13}C NMR: δ 163.5, 161.6, 159.7, 158.8, 152.6, 148.4, 147.9, 138.4, 126.0, 119.1, 118.3, 115.0, 47.7, 32.0, 19.6, 17.9. HRMS (ESI). Calcd for $C_{16}H_{14}N_4O_2S$ [M–H]$^-$: *m/z* 326.0833. Found: *m/z* 326.0837.

2-Methyl-*N*-(4-methylpyridin-2-yl)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (9q) Yield 92%; light yellow solid; mp 166–168°C; ^{1}H NMR: δ 13.32 (s, 1H), 8.21 (d, *J* = 5 Hz, 1H), 8.18 (s, 1H), 6.81 (d, *J* = 5 Hz, 1H), 4.27 (t, *J* = 7 Hz, 2H), 3.16 (t, *J* = 7 Hz, 2H), 2.92 (s, 3H), 2.23–2.11 (m, 2H); ^{13}C NMR: δ 162.3, 161.4, 159.8, 156.2, 152.8, 148.6, 147.8, 146.9, 126.3, 119.7, 118.2, 115.0, 46.3, 31.9, 22.4, 19.1, 18.0. HRMS (ESI). Calcd for $C_{17}H_{16}N_4O_2S$ [M–H]$^-$: *m/z* 326.0843. Found: m/z 340.0894.

***N*-Butyl-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10a)** Yield 79%; white solid; mp 209–211°C; ^{1}H NMR: δ 10.40 (d, *J* = 4 Hz, 1H), 4.23 (t, *J* = 7 Hz, 2H), 3.41 (t, *J* = 7 Hz, 2H), 3.20 (t, *J* = 8 Hz, 2H), 2.87 (s, 3H), 2.12–2.06 (m, 2H), 1.97–1.91 (m, 2H)., 1.67–1.57 (m, 2H), 1.48–1.38 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR: δ 163.4, 163.3, 159.2, 158.5, 145.8, 126.3, 118.6, 47.3, 39.4, 31.9, 31.4, 21.2, 20.4, 19.6, 17.5, 13.9. HRMS (ESI). Calcd for $C_{16}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 319.1350. Found: *m/z* 319.1354.

***N*-(*sec*-Butyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10b)** Yield 86%; light yellow solid; mp 109–111°C; ^{1}H NMR: δ 10.19 (d, *J* = 6 Hz, 1H), 4.07 (t, *J* = 6 Hz, 2H), 4.08–4.02 (m, 1H), 2.99 (t, *J* = 7 Hz, 2H), 2.86 (s, 3H), 2.06–2.01 (m, 2H), 1.97–1.91 (m, 2H), 1.70–1.56 (m, 2H), 1.25 (d, *J* = 7 Hz, 3H), 0.98 (t, *J* = 7 Hz, 3H); ^{13}C NMR: δ 162.7, 161.6, 159.6, 154.8, 145.1, 126.8, 118.5, 46.7, 43.1, 31.5, 29.5, 22.1, 20.1, 19.0, 17.4, 10.6. HRMS (ESI). Calcd for $C_{16}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 319.1350. Found: *m/z* 319.1354.

***N*,*N*-Diisopropyl-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido-[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10c)** Yield 89%; light yellow solid; mp 187–189°C; ^{1}H NMR: δ 4.13–4.04 (m, 1H), 3.98–3.91 (m, 1H), 3.79–3.72 (m, 1H), 3.57–3.49 (m, 1H), 2.96 (t, *J* = 6 Hz, 2H), 2.44 (s, 3H), 2.01–1.89 (m, 4H), 1.68 (d, *J* = 7 Hz, 3H), 1.58 (d, *J* = 7 Hz, 3H), 1.13 (dd, *J* = 7 Hz, 4 Hz, 6H); ^{13}C NMR: δ 165.0, 162.1, 157.2, 155.3, 131.2, 130.9, 119.7, 51.2, 45.9, 42.1, 31.6, 22.0, 21.3, 20.8, 20.2, 19.9, 19.2, 13.7. HRMS (ESI). Calcd for $C_{18}H_{25}N_3O_2S$ [M–H]$^-$ *m/z* 347.1661. Found: *m/z* 347.1667.

2-Methyl-*N*-octyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (10d) Yield 81%; light yellow solid; mp 103–105°C; ^{1}H NMR: δ 10.42 (s, 1H), 4.06 (t, *J* = 6 Hz, 2H), 3.40 (dd, *J* = 12 Hz, 7 Hz, 2H), 3.00 (t, *J* = 7 Hz, 2H), 2.86 (s, 3H), 2.06–2.02 (m, 2H), 1.98–1.90 (m, 2H), 1.68–1.60 (m, 2H), 1.44–1.25 (m, 10H), 0.88 (t, *J* = 7 Hz, 3H); ^{13}C NMR: δ 163.4, 161.7, 159.6, 154.7, 145.4, 126.5, 118.5, 43.1, 39.7, 31.8, 31.5, 29.4, 29.3, 29.2, 27.2, 22.7, 22.2, 19.0, 17.4, 14.1. HRMS (ESI). Calcd for $C_{20}H_{29}N_3O_2S$ [M–H]$^-$: *m/z* 375.1986. Found: *m/z* 375.1980.

***N*-Cyclohexyl-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10e)** Yield 77%; white solid; mp 195–197°C; ^{1}H NMR: δ 10.23 (d, *J* = 7 Hz, 1H), 4.07 (t, *J* = 6 Hz, 2H), 3.97 –3.90 (m, 1H), 3.00 (t, *J* = 7 Hz, 2H), 2.85 (s, 3H), 2.06 –1.98 (m, 4H), 1.98–1.91 (m, 2H), 1.81–1.74 (m, 2H), 1.48–1.20 (m, 6H); ^{13}C NMR: δ 162.4, 161.4, 159.5, 154.8, 145.2, 126.8, 118.5, 48.4, 43.1, 32.9, 31.4, 25.8, 25.0, 22.1, 19.0, 17.4. HRMS (ESI). Calcd for $C_{18}H_{23}N_3O_2S$ [M–H]$^-$: *m/z* 345.1517. Found: *m/z* 345.1511.

2-Methyl-4-oxo-*N*-(*o*-tolyl)-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (10f) Yield 80%; light yellow solid; mp 155–157°C; ^{1}H NMR: δ 12.18 (s, 1H), 7.78 (d, *J* = 8 Hz, 1H), 7.25–7.18 (m, 2H), 7.12–7.05 (m, 1H), 4.09 (t, *J* = 6 Hz, 2H), 3.02 (t, *J* = 7 Hz, 2H), 2.91 (s, 3H), 2.38 (s, 3H), 2.07–2.01 (m, 2H), 1.99–1.91 (m, 2H). ^{13}C NMR: δ 161.7, 161.6, 159.8, 154.9, 147.4, 136.6, 131.6, 130.5, 126.2, 126.9, 125.1, 124.9, 118.3, 43.2, 31.5, 22.1, 19.0, 18.5, 17.9. HRMS (ESI). Calcd for $C_{19}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 353.1191. Found: *m/z* 353.1198.

2-Methyl-4-oxo-*N*-(*p*-tolyl)-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]-thieno[2,3-*d*]pyrimidine-3-carboxamide (10g) Yield 75%; light yellow solid, mp 226–228°C; ^{1}H NMR: δ 12.81 (s, 1H), 7.67 (d, *J* = 8 Hz, 2H), 7.14 (d, *J* = 8 Hz, 2H), 4.09 (t, *J* = 6 Hz, 2H), 3.01 (t, *J* = 7 Hz, 2H), 2.92 (s, 3H), 2.33 (s, 3H), 2.07–2.01 (m, 2H), 1.98–1.90 (m, 2H); ^{13}C NMR: δ 161.6, 161.1, 160.1, 154.9, 147.2, 136.7, 133.1, 129.3, 126.5, 120.3, 118.1, 43.4, 31.5, 22.2, 20.9, 19.0, 17.9. HRMS (ESI). Calcd for $C_{19}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 353.1193. Found: *m/z* 353.1198.

***N*-(4-Methoxyphenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10h)** Yield 86%; light yellow solid; mp 201–203°C; ^{1}H NMR: δ 12.74

(s, 1H), 7.70 (d, *J* = 9 Hz, 2H), 6.89 (d, *J* = 9 Hz, 2H), 4.10 (t, *J* = 6 Hz, 2H), 3.81 (s, 3H), 3.03 (t, *J* = 7 Hz, 2H), 2.92 (s, 3H), 2.08–2.02 (m, 2H), 2.00–1.92 (m, 2H); ^{13}C NMR: δ 161.4, 161.0, 160.0, 155.9, 154.9, 147.1, 132.4, 126.4, 121.8, 118.1, 114.0, 55.5, 43.4, 31.4, 22.1, 19.0, 17.8. HRMS (ESI). Calcd for $C_{19}H_{19}N_3O_3S$ [M–H]$^-$: *m/z* 369.1142. Found: *m/z* 369.1147.

***N*-(2,6-Dimethylphenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10i)** Yield 83%; white solid; mp 186–188°C; ^{1}H NMR: δ 12.11 (s, 1H), 7.12–7.04 (m, 3H), 4.08 (t, *J* = 6 Hz, 2H), 3.03 (t, *J* = 6 Hz, 2H), 2.91 (s, 3H), 2.31 (s, 6H), 2.08–2.01 (m, 2H), 2.00–1.92 (m, 2H); ^{13}C NMR: δ 161.8, 161.6, 159.9, 154.9, 147.3, 135.3, 135.2, 127.9, 126.5, 125.8, 118.4, 43.3, 31.5, 22.1, 19.0, 18.7, 17.7. HRMS (ESI). Calcd for $C_{20}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 367.1360. Found: *m/z* 367.1354.

***N*-(4-Acetylphenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10j)** Yield 74%; white solid; mp 230–232°C; ^{1}H NMR: δ 13.35 (s, 1H), 7.95 (d, *J* = 9 Hz, 2H), 7.89 (d, *J* = 9 Hz, 2H), 4.31 (t, *J* = 7 Hz, 2H), 3.25 (t, *J* = 8 Hz, 2H), 2.91 (s, 3H), 2.62 (s, 3H), 2.45–2.37 (m, 2H). 2.05–1.93 (m, 2H); ^{13}C NMR: δ 197.1, 162.1, 160.2, 159.8, 159.6, 149.3, 143.8, 132.3, 129.5, 126.0, 119.5, 118.0, 45.6, 31.7, 26.6, 22.7, 19.3, 18.1. HRMS (ESI). Calcd for $C_{20}H_{19}N_3O_3S$ [M–H]$^-$: *m/z* 381.1154. Found: *m/z* 381.1147.

***N*-(4-Chlorophenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10k)** Yield 83%; light yellow solid; mp 238–240°C; ^{1}H NMR: δ 13.15 (s, 1H), 7.76 (d, *J* = 9 Hz, 2H), 7.30 (d, *J* = 9 Hz, 2H), 4.12 (t, *J* = 6 Hz, 2H), 3.03 (t, *J* = 6 Hz, 2H), 2.93 (s, 3H), 2.11–2.03 (m, 2H), 2.01–1.93 (m, 2H); ^{13}C NMR: δ 161.8, 161.2, 160.2, 154.9, 148.1, 137.9, 128.7, 128.3, 126.0, 121.4, 117.9, 43.5, 31.5, 22.1, 19.0, 18.0. HRMS (ESI). Calcd for $C_{18}H_{16}ClN_3O_2S$ [M–H]$^-$: *m/z* 373.0646. Found: *m/z* 373.0652.

***N*-(2-Chlorophenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10l)** Yield 87%; light yellow solid; mp 182–184°C; ^{1}H NMR: δ 12.08 (s, 1H), 8.15 (d, *J* = 8 Hz, 1H), 7.42 (dd, *J* = 8, 1.1 Hz, 1H), 7.34–7.27 (m, 1H), 7.09 (td, *J* = 8 Hz, 1.4 Hz, 1H), 4.11 (t, *J* = 6 Hz, 2H), 3.03 (t, *J* = 6 Hz, 2H), 2.88 (s, 3H), 2.08–2.02 (m, 2H), 1.99–1.92 (m, 2H); ^{13}C NMR: δ 161.8, 161.6, 159.5, 155.1, 147.2, 135.4, 127.1, 126.4, 126.0, 125.4, 125.2, 118.3, 43.2, 31.5, 22.1, 19.0, 17.6. HRMS (ESI). Calcd for $C_{18}H_{16}ClN_3O_2S$ [M–H]$^-$: *m/z* 373.0645. Found: *m/z* 373.0652.

***N*-(4-Bromophenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10m)** Yield 94%; white solid; mp 234–236°C; ^{1}H NMR: δ 13.16 (s, 1H), 7.70 (d, *J* = 9 Hz, 2H), 7.44 (d, *J* = 9 Hz, 2H), 4.10 (t, *J* = 6 Hz, 2H), 3.03 (t, *J* = 6 Hz, 2H), 2.92 (s, 3H), 2.09–2.03 (m, 2H), 2.01–1.93 (m, 2H); ^{13}C NMR: δ 161.8, 161.2, 160.2, 154.9, 148.1, 138.4, 131.6, 126.0, 121.7, 117.9, 116.0, 77.3, 77.0, 76.7, 43.5, 31.5, 22.1, 19.0, 18.0. HRMS (ESI). Calcd for $C_{18}H_{16}BrN_3O_2S$ [M–H]$^-$: *m/z* 417.0145. Found: *m/z* 417.0147.

***N*-(2,6-Dichlorophenyl)-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10n)** Yield 91%; light yellow solid; mp 165–167°C; ^{1}H NMR: δ 12.26 (s, 1H), 8.14 (d, *J* = 9 Hz, 1H), 7.42 (d, *J* = 2.3 Hz, 1H), 7.29–7.22 (m, 1H), 4.11 (t, *J* = 6 Hz, 2H), 3.03 (t, *J* = 6 Hz, 2H), 2.88 (s, 3H), 2.08–2.01 (m, 2H), 2.00–1.92 (m, 2H); ^{13}C NMR: δ 161.8, 161.4, 159.6, 155.2, 147.9, 134.4, 129.7, 129.2, 127.2, 126.9, 125.8, 125.7, 118.2, 43.3, 31.5, 22.1, 19.0, 17.7. HRMS (ESI). Calcd for $C_{18}H_{15}Cl_2N_3O_2S$ [M–H]$^-$: *m/z* 407.0260. Found: *m/z* 407.0262.

***N*-Benzyl-2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10o)** Yield 82%; white solid; mp 113–115°C; ^{1}H NMR: δ 10.90 (s, 1H), 7.41 (d, *J* = 7 Hz, 2H), 7.32 (t, *J* = 7 Hz, 2H), 7.24 (t, *J* = 7 Hz, 1H), 4.64 (d, *J* = 5 Hz, 2H), 4.03 (t, *J* = 6 Hz, 2H), 3.00 (t, *J* = 6 Hz, 2H), 2.87 (s, 3H), 2.04–1.97 (m, 2H), 1.97–1.90 (m, 2H); ^{13}C NMR: δ 163.4, 161.4, 159.6, 154.9, 146.0, 139.0, 128.4, 127.7, 126.9, 126.1, 118.4, 43.5, 43.2, 31.4, 22.1, 19.0, 17.5. HRMS (ESI). Calcd for $C_{19}H_{19}N_3O_2S$ [M–H]$^-$: *m/z* 353.1203. Found: *m/z* 353.1198.

2-Methyl-4-oxo-*N*-(pyridin-2-yl)-6,7,8,9-tetrahydro-4*H*-pyrido-[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10p) Yield 75%; white solid; mp 181–183°C; ^{1}H NMR: δ 13.29 (s, 1H), 8.31 (dd, *J* = 5 Hz, 1.2 Hz, 1H), 8.27 (d, *J* = 8 Hz, 1H), 7.67–7.59 (m, 1H), 7.11–7.05 (m, 1H), 4.31 (t, *J* = 7 Hz, 2H), 3.13 (t, *J* = 8 Hz, 2H), 2.94 (s, 3H), 2.08–2.04 (m, 2H), 1.97–1.91 (m, 2H); ^{13}C NMR: δ 162.7, 161.9, 159.8, 156.7, 152.4, 149.2, 147.9, 138.9, 126.2, 119.8, 118.6, 115.7, 47.7, 32.0, 19.6, 17.9. HRMS (ESI). Calcd for $C_{17}H_{16}N_4O_2S$ [M–H]$^-$: *m/z* 340.0988. Found: *m/z* 340.0994.

2-Methyl-*N*-(4-methylpyridin-2-yl)-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]thieno[2,3-*d*]pyrimidine-3-carboxamide (10q) Yield 84%; white solid; mp 190–192°C; ^{1}H NMR: δ 13.29 (s, 1H), 8.28 (d, *J* = 5 Hz, 1H), 8.24 (s, 1H), 6.85 (d, *J* = 5 Hz, 1H), 4.19 (t, *J* = 6 Hz, 2H), 3.01 (t, *J* = 6 Hz, 2H), 2.93 (s, 3H), 2.39 (s, 3H), 2.06–1.99 (m, 2H), 1.98–1.91 (m, 2H); ^{13}C NMR: δ 161.7, 161.6, 159.9, 155.2, 152.8, 149.4, 148.0, 147.9, 126.2, 120.4, 118.1, 115.3, 43.4, 31.5, 22.1, 21.4, 19.0, 17.9. HRMS (ESI). Calcd for $C_{18}H_{18}N_4O_2S$ [M–H]$^-$: *m/z* 354.1154. Found: *m/z* 354.1150.

***N*-Butyl-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxamide (11a)** Yield 75%; white solid; mp 121–123°C; ^{1}H NMR: δ 10.18 (s, 1H), 4.40 (t, *J* = 4 Hz, 2H), 3.41 (q, *J* =5 Hz, 2H), 3.07 (t, *J* = 5 Hz, 2H), 2.85 (s, 3H), 1.84 (dd, *J* = 20 Hz, 2.6 Hz, 6H), 1.68–1.59 (m, 2H), 1.49–1.39 (m, 2H), 0.96 (t, *J* = 7 Hz, 3H); ^{13}C NMR: δ 163.4, 161.1, 159.6, 159.2, 145.5, 126.8, 118.5, 43.3, 39.4, 37.1, 31.5, 29.4, 27.4, 25.0, 20.4, 17.3, 13.9. HRMS (ESI). Calcd for $C_{17}H_{23}N_3O_2S$ [M–H]$^-$: *m/z* 333.1516. Found: *m/z* 333.1511.

***N*-(*sec*-Butyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydro-thieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11b)** Yield 82%; light yellow solid; mp 153–155°C; ^{1}H NMR: δ 9.96 (s, 1H), 4.41 (t, *J* = 4 Hz, 2H), 3.06 (t, *J* = 4 Hz, 2H), 2.84 (s, 3H), 1.87–1.81 (m, 6H), 1.69–1.54 (m, 2H), 1.25 (d, *J* = 7 Hz, 3H), 0.98 (t, *J* = 7 Hz, 3H); ^{13}C NMR: δ 162.8, 161.2, 159.6, 159.2, 145.1, 127.2, 118.6, 46.8, 43.3, 37.1, 29.5, 29.5, 27.5, 25.0, 20.1, 17.3, 10.6. HRMS (ESI). Calcd for $C_{17}H_{23}N_3O_2S$ [M–H]$^-$: *m/z* 333.1518. Found: *m/z* 333.1511.

***N*,*N*-Diisopropyl-2-methyl-4-oxo-4,6,7,8,9,10-hexahydro-thieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11c)** Yield 80%; light yellow solid; mp 178–180°C; ^{1}H NMR: δ 4.45 (t, *J* = 4 Hz, 2H), 3.79–3.69 (m, 1H), 3.58–3.48 (m, 1H), 3.06 (t, *J* = 4 Hz, 2H), 2.43 (s, 3H), 1.93–1.70 (m, 6H), 1.68 (d, *J* = 7 Hz, 3H), 1.58 (d, *J* =7Hz, 3H), 1.13 (t, *J* = 6 Hz, 6H); ^{13}C NMR: δ 164.9, 161.4, 160.2, 156.9, 131.8, 131.2, 119.7, 51.2, 45.9, 42.4, 37.3, 29.6, 27.7, 25.0, 21.2, 20.8, 20.1, 20.0, 13.7. HRMS (ESI). Calcd for $C_{19}H_{27}N_3O_2S$ [M–H]$^-$: *m/z* 361.1830. Found: *m/z* 361.1824.

2-Methyl-*N*-octyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11d) Yield 84%; light yellow solid; mp 102–104°C; ^{1}H NMR: δ 10.18 (s, 1H), 4.40 (t, *J* = 4 Hz,

2H), 3.42–3.37 (m, 2H), 3.06 (t, *J* = 4 Hz, 2H), 2.85 (s, 3H), 1.82–1.78 (m, 6H), 1.68–1.59 (m, 2H), 1.44–1.21 (m, 10H), 0.87 (t, *J* = 6 Hz, 3H); ^{13}C NMR: δ 163.4, 161.4, 159.6, 159.3, 145.5, 126.8, 118.5, 43.3, 39.7, 37.1, 31.8, 29.5, 29.4, 29.3, 29.2, 27.5, 27.2, 25.0, 22.7, 17.4, 14.1. HRMS (ESI). Calcd for $C_{21}H_{31}N_3O_2S$ [M–H]$^-$: *m/z* 389.2132. Found: *m/z* 389.2137.

***N*-Cyclohexyl-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11e)** Yield 76%; light yellow solid; mp 186–188°C; ^{1}H NMR: δ 10.06 (d, *J* = 5 Hz, 1H), 4.41 (t, *J* = 4 Hz, 2H), 3.06 (t, *J* = 4 Hz, 2H), 2.83 (s, 3H), 2.04–2.04 (m, 2H), 1.86–1.76 (m, 8H), 1.65–1.62 (m, 1H), 1.50–1.16 (m, 6H); ^{13}C NMR: δ 162.5, 161.3, 159.6, 159.2, 145.2, 127.1, 118.5, 48.4, 43.2, 37.2, 32.9, 29.5, 27.5, 25.8, 25.0, 25.0, 17.3. HRMS (ESI). Calcd for $C_{19}H_{25}N_3O_2S$ [M–H]$^-$: *m/z* 359.1663. Found: *m/z* 359.1667.

2-Methyl-4-oxo-*N*-(*o*-tolyl)-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11f) Yield 86%; light yellow solid; mp 191–193°C; ^{1}H NMR: δ 12.06 (s, 1H), 7.78 (d, *J* = 8 Hz, 1H), 7.22 (dt, *J* = 7 Hz, 4 Hz, 2H), 7.08 (t, *J* = 7 Hz, 1H), 4.48–4.36 (m, 2H), 3.09 (s, 2H), 2.90 (s, 3H), 2.37 (s, 3H), 1.85 (m, 6H); ^{13}C NMR: δ 161.7, 161.3, 159.7, 159.5, 147.5, 136.5, 131.6, 130.4, 126.5, 126.2, 125.1, 124.8, 118.3, 43.4, 37.1, 29.4, 27.4, 25.0, 18.4, 17.8. HRMS (ESI). Calcd for $C_{20}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 367.1359. Found: *m/z* 367.1354.

2-Methyl-4-oxo-*N*-(*p*-tolyl)-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11g) Yield 90%; light yellow solid; mp 223–225°C; ^{1}H NMR: δ 12.71 (s, 1H), 7.67 (d, *J* = 8 Hz, 2H), 7.15 (d, *J* = 8 Hz, 2H), 4.56 (t, *J* = 4 Hz, 2H), 3.09 (d, *J* = 5 Hz, 2H), 2.93 (s, 3H), 2.33 (s, 3H), 1.96–1.61 (m, 6H); ^{13}C NMR: δ 161.4, 161.1, 159.8, 159.7, 147.5, 136.6, 133.1, 129.3, 126.8, 120.3, 118.2, 43.5, 37.1, 29.4, 27.4, 25.0, 20.9, 17.9. HRMS (ESI). Calcd for $C_{20}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 367.1360. Found: *m/z* 367.1354.

***N*-(4-Methoxyphenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11h)** Yield 79%; white solid; mp 200–202°C; ^{1}H NMR: δ 12.67 (s, 1H), 7.70 (d, *J* = 9 Hz, 2H), 6.89 (d, *J* = 9 Hz, 2H), 4.56 (t, *J* = 4 Hz, 2H), 3.81 (s, 3H), 3.09 (t, *J* = 5 Hz, 2H), 2.92 (s, 3H), 1.94–1.60 (m, 6H); ^{13}C NMR: δ 161.3, 161.0, 159.8, 159.7, 156.0, 147.4, 132.4, 126.7, 121.9, 118.2, 114.0, 55.5, 43.5, 37.1, 29.4, 27.4, 25.0, 17.9. HRMS (ESI). Calcd for $C_{20}H_{21}N_3O_3S$ [M–H]$^-$: *m/z* 383.1310. Found: *m/z* 383.1304.

***N*-(2,6-Dimethylphenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11i)** Yield 86%; white solid; mp 199–201°C; ^{1}H NMR: δ 11.93 (s, 1H), 7.12–7.04 (m, 3H), 4.45 (t, *J* = 4 Hz, 2H), 3.12 (t, *J* = 4 Hz, 2H), 2.90 (s, 3H), 2.31 (s, 6H), 1.91–1.82 (m, 6H); ^{13}C NMR: δ 161.6, 161.4, 159.7, 159.6, 147.6, 135.3, 135.2, 127.9, 126.5, 126.1, 118.5, 43.5, 37.1, 29.4, 27.4, 25.0, 18.7, 17.8. HRMS (ESI). Calcd for $C_{21}H_{23}N_3O_2S$ [M–H]$^-$: *m/z* 381.1506. Found: *m/z* 381.1511.

***N*-(4-Acetylphenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11j)** Yield 88%; light yellow solid; mp 203–205°C; ^{1}H NMR: δ 13.40 (s, 1H), 7.97 (d, *J* = 8 Hz, 2H), 7.90 (d, *J* = 8 Hz, 2H), 4.50 (t, *J* = 4 Hz, 2H), 3.11 (t, *J* = 4 Hz, 2H), 2.95 (s, 3H), 2.59 (s, 3H), 1.92–1.81 (m, 6H); ^{13}C NMR: δ 197.2, 161.6, 161.4, 160.0, 159.8, 149.0, 143.9, 132.2, 129.6, 126.1, 119.4, 118.0, 43.6, 37.2, 29.4, 27.4, 26.4, 25.0, 18.1. HRMS (ESI). Calcd for $C_{21}H_{21}N_3O_3S$ [M–H]$^-$: *m/z* 395.1300. Found: *m/z* 395.1304.

***N*-(4-Chlorophenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11k)** Yield 82%; light yellow solid; mp 253–255°C; ^{1}H NMR: δ 13.05 (s, 1H), 7.75 (d, *J* = 8 Hz, 2H), 7.30 (d, *J* = 8 Hz, 2H), 4.47 (t, *J* = 5 Hz, 2H), 3.10 (t, *J* = 5 Hz, 2H), 2.92 (s, 3H), 2.01–1.76 (m, 6H); ^{13}C NMR: δ 161.6, 161.2, 160.0, 159.7, 148.3, 137.9, 128.7, 128.4, 126.3, 121.5, 118.0, 43.6, 37.1, 29.4, 27.4, 25.0, 18.0. HRMS (ESI). Calcd for $C_{19}H_{18}ClN_3O_2S$ [M–H]$^-$: *m/z* 387.0810. Found: *m/z* 387.0808.

***N*-(2-Chlorophenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11l)** Yield 76%; white solid; mp 173–175°C; ^{1}H NMR: δ 13.08 (s, 1H), 8.21 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8, 1H), 7.31–7.24 (m, 1H), 7.12 (t, *J* = 8, Hz, 1H), 4.26 (t, *J* = 6 Hz, 2H), 3.13 (t, *J* = 6 Hz, 2H), 2.93 (s, 3H), 2.08–1.89 (m, 6H); ^{13}C NMR: δ 161.8, 161.6, 159.6, 155.3, 147.2, 135.4, 127.2, 126.5, 126.1, 125.5, 125.2, 118.2, 43.7, 37.1, 29.4, 27.4, 24.9, 18.1. HRMS (ESI). Calcd for $C_{19}H_{18}ClN_3O_2S$ [M–H]$^-$: *m/z* 387.0812. Found: *m/z* 387.0808.

***N*-(4-Bromophenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11m)** Yield 84%; light yellow solid; mp 263–265°C; ^{1}H NMR: δ 13.08 (s, 1H), 7.71 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8 Hz, 2H), 4.47 (t, *J* = 5 Hz, 2H), 3.10(t, *J* = 5 Hz, 3H), 2.93 (s, 5H), 1.93–1.83 (m, 6H); ^{13}C NMR: δ 161.7, 161.2, 160.0, 159.7, 148.4, 138.4, 131.7, 126.3, 121.8, 118.0, 116.0, 43.6, 37.2, 29.4, 27.4, 25.0, 18.0. HRMS (ESI). Calcd for $C_{19}H_{18}BrN_3O_2S$ [M–H]$^-$: *m/z* 431.0308. Found: *m/z* 431.0303.

***N*-(2,4-Dichlorophenyl)-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11n)** Yield 91%; light yellow solid; mp 201–203°C; ^{1}H NMR: δ 12.11 (s, 1H), 8.16 (d, *J* = 9 Hz, 1H), 7.42 (d, *J* = 4 Hz, 1H), 7.25 (t, *J* = 4 Hz, 1H), 4.46 (t, *J* = 4 Hz, 2H), 3.12–3.05 (t, *J* = 4 Hz, 2H), 2.87 (s, 3H), 1.91–1.79 (m, 6H); ^{13}C NMR: δ 161.6, 161.1, 159.4, 155.2, 147.7, 134.5, 129.7, 129.2, 127.2, 126.8, 125.8, 125.8, 118.2, 43.5, 37.2, 29.3, 27.4, 25.0, 18.1. HRMS (ESI). Calcd for $C_{19}H_{17}Cl_2N_3O_2S$ [M–H]$^-$: *m/z* 421.0423. Found: *m/z* 421.0419.

***N*-Benzyl-2-methyl-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11o)** Yield 73%; light yellow solid; mp 116–118°C; ^{1}H NMR: δ 10.76 (s, 1H), 7.41 (d, *J* = 8 Hz, 2H), 7.33 (t, *J* = 8 Hz, 2H), 7.24 (t, *J* = 8 Hz, 1H), 4.64 (d, *J* = 5 Hz, 2H), 4.41–4.34 (t, *J* = 5 Hz, 2H), 3.06 (t, *J* = 5 Hz, 2H), 2.86 (s, 3H), 1.96–1.61 (m, 6H); ^{13}C NMR: δ 163.5, 161.4, 159.6, 159.3, 146.2, 139.0, 128.4, 127.7, 126.9, 126.4, 118.5, 43.4, 43.3, 37.1, 29.4, 27.4, 25.0, 17.5. HRMS (ESI). Calcd for $C_{20}H_{21}N_3O_2S$ [M–H]$^-$: *m/z* 367.1350. Found: *m/z* 367.1354.

2-Methyl-4-oxo-*N*-(pyridin-2-yl)-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11p) Yield 79%; light yellow solid; mp 212–214°C; ^{1}H NMR: δ 13.29 (s, 1H), 8.44 (d, *J* = 4 Hz, 1H), 8.40 (d, *J* = 8 Hz, 1H), 7.75–7.68 (m, 1H), 7.03 (dd, *J* = 7 Hz, 5 Hz, 1H), 4.53 (t, *J* = 4 Hz, 2H), 3.08 (t, *J* = 4 Hz, 2H), 2.92 (s, 3H), 1.87–1.81 (m, 6H); ^{13}C NMR: δ 161.7, 161.5, 160.0, 159.7, 152.7, 148.3, 148.2, 138.1, 126.3, 119.1, 118.1, 114.9, 43.5, 37.2, 29.5, 27.7, 25.0, 17.9. HRMS (ESI). Calcd for $C_{18}H_{18}N_4O_2S$ [M–H]$^-$: *m/z* 354.1156. Found: *m/z* 354.1150.

2-Methyl-*N*-(4-methylpyridin-2-yl)-4-oxo-4,6,7,8,9,10-hexahydrothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxamide (11q) Yield 74%; light yellow solid; mp 237–239°C; ^{1}H NMR: δ 13.21 (s, 1H), 8.27 (d, *J* = 9 Hz, 2H), 6.86 (d, *J* = 4 Hz, 1H), 4.55 (t, *J* = 4 Hz, 2H), 3.08 (t, *J* = 5 Hz, 2H), 2.92 (s, 3H), 2.39 (s, 3H), 1.86–1.81 (m, 6H); ^{13}C NMR: δ 161.7, 161.5, 160.0, 159.7, 152.7, 149.5, 148.1, 147.9, 126.4, 120.4,

118.2, 115.3, 43.5, 37.2, 29.5, 27.7, 25.0, 21.5, 18.0. HRMS (ESI). Calcd for $C_{19}H_{20}N_4O_2S$ $[M-H]^-$: *m/z* 368.1315. Found: *m/z* 368.1307.

Cell culture

Murine B16 melanoma cell lines (B16F10) were obtained from the Chinese Academy of Sciences. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (GIBICO, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBICO, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (GIBCO, USA) in a humidified atmosphere with 5% CO_2 at 37°C.

Melanin content assay

Exponentially growing cells were seeded into 6-well plates at a concentration of 5×10^5 cells per well. After 24 h of incubation at 37°C, the culture medium was removed and replaced with fresh medium containing the candidate compounds at different concentrations. The cells were incubated for another 48 h, washed with ice-cold phosphate-buffered saline (PBS), followed by lysis with radioimmunoprecipitation assay (RIPA) buffer for 40 min on ice, and the lysates were centrifuged at 10 000 *g* for 20 min. Supernatants containing protein were subjected to protein assay, and the pellets with intracellular melanin were solubilized in 200 μL of 1 M NaOH for 2 h at 60°C. The melanin amount was determined spectrophotometrically at 405 nm using a multi-plate reader. The melanin amount was calculated by normalizing the total melanin values with protein content (abs melanin/L g protein).

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