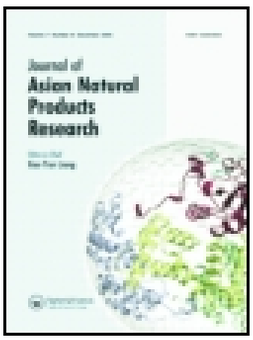


Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

Straightforward synthesis, characterization, and cytotoxicity evaluation of hybrids of natural alkaloid evodiamine/rutaecarpine and thieno[2,3-*d*]pyrimidinones

Li-Fei Nie, Si-Si Wang, Jian-Guo Cao, Fei-Ze Liu, Hainimu Xiamuxi, Haji Akber Aisa & Guo-Zheng Huang

To cite this article: Li-Fei Nie, Si-Si Wang, Jian-Guo Cao, Fei-Ze Liu, Hainimu Xiamuxi, Haji Akber Aisa & Guo-Zheng Huang (2018): Straightforward synthesis, characterization, and cytotoxicity evaluation of hybrids of natural alkaloid evodiamine/rutaecarpine and thieno[2,3-*d*]pyrimidinones, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2018.1540599

To link to this article: https://doi.org/10.1080/10286020.2018.1540599

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2018.1540599

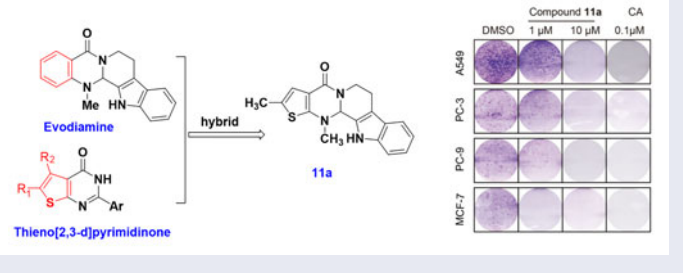
Straightforward synthesis, characterization, and cytotoxicity evaluation of hybrids of natural alkaloid evodiamine/rutaecarpine and thieno[2,3-*d*]pyrimidinones

Li-Fei Nie[a,b]*, Si-Si Wang[c]*, Jian-Guo Cao[c], Fei-Ze Liu[a,b], Hainimu Xiamuxi[a,b], Haji Akber Aisa[a] and Guo-Zheng Huang[a,c]

[a]Key Laboratory of Plant Resources and Chemistry of Arid Zone, State Key Laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, China; [b]University of Chinese Academy of Sciences, Beijing 100049, China; [c]College of Life and Environmental Sciences, Shanghai Normal University, Shanghai 201418, China

ABSTRACT

Dozens of hybrids of natural alkaloid evodiamine/rutaecarpine and thieno[2,3-*d*]pyrimidinones were synthesized in a straightforward method by condensation of substituted 2*H*-thieno[2,3-*d*][1, 3]oxazine-2,4(1*H*)-diones or *N*-methyl-2*H*-thieno[2,3-*d*][1, 3]oxazine-2,4(1*H*)-dione with 3,4-dihydro-β-carbolines. *In vitro* cytotoxic assay discovered that compounds **9a**, **10e**, **11a**, **11d**, **11f**, and **12a** could induce antiproliferation against four different types of human cancer cells while compounds **10f** and **12e** were inactive. Notably, compound **11a** displayed potent cell cytotoxicity for human non-small cell lung cancer cells A549, PC-9, human prostate cancer cells PC-3, and human breast cancer cell line MCF-7. Furthermore, compound **11a** exhibited strong colony formation inhibition to A549 cells. These results unfold potential anticancer therapeutic applications of hybrids of thieno[2,3-*d*]pyrimidinones and quinazolinones.



ARTICLE HISTORY
Received 15 June 2018
Revised 18 October 2018
Accepted 18 October 2018

KEYWORDS
Thieno[2,3-*d*]pyrimidinone; evodiamine; rutaecarpine; hybrid; cytotoxicity

CONTACT Haji Akber Aisa haji@ms.xjb.ac.cn; Guo-Zheng Huang g.huang@ms.xjb.ac.cn Key Laboratory of Plant Resources and Chemistry of Arid Zone, State Key Laboratory Basis of Xinjiang Indigenous Medicinal Plants Resource Utilization, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, China.
*These authors contribute equally.

Supplemental data for this article can be accessed at https://doi.org/10.1080/10286020.2018.1540599.

1. Introduction

Quinazolinones are generally regarded as one of the privileged pharmacophores for drug discovery. Application of quinazolinone skeleton could be found in drug candidates for the evaluation of diverse biological activities including antimicrobial, anti-inflammatory, antimalarial, antiviral, and last but not least anti-cancer activities [1–4]. Among the two structural isomers, 4-quinazolinones are far more abundantly occurring in synthetic and natural products than 2- quinazolinones [5].

Evodiamine and rutaecarpine (compounds **1** and **2**, Figure 1) are two main 4-quinazolinone alkaloids isolated from the fruit of the plant *Tetradium ruticarpum*, which is used in traditional Chinese medicine for the treatment of a variety of diseases. Although their isolation was achieved nearly a century ago, they are still being actively investigated as leads for drug developments [6], such as for anti-influenza [7], anti-Alzheimers [8, 9], anti-cancer [10], and vasodilator effect [11]. Wang et al. [12, 13] recently modified evodiamine to obtain a series of highly potent and multi-targeting antitumor agents. Rutaecarpine was reported for its effect of antiproliferative [14], anti-hyperlipidemia and hyperglycemia [15] and protective effect of hepatotoxicity [16]. Incorporation of evodiamine or rutaecarpine into hybrids was also been investigated [17, 18].

Molecular hybridization has been widely applied for drug discovery in recent years. This strategy usually combined two active compounds with or without a linker to obtain a new drug identity with potential synergistic or even over-additive effect [19]. Alternatively, two compounds can be fused together by replacement the similar moiety in one compound with that of the other compound. Thieno[2,3-*d*]pyrimidin-4(3*H*)-one is a favored building block commonly applied for the drug development with diversified pharmaceutical activities. It has been widely used for preparation of new compounds as antibacterial, antitumor agents, etc. Compounds **3** and **4** (Figure 1) represented two good examples of derivative of thieno[2,3-*d*]pyrimidin-4(3*H*)-one as potent anticancer agents [20, 21]. As part of our continuous research in the synthesis of new evodiamine and rutaecarpine derivatives [22, 23], herein we present our application of molecular hybridization strategy to synthesize dozen of hybrids of thieno[2,3-*d*]pyrimidin-4(3*H*)-one and evodiamine/rutaecarpine and to evaluate their cytotoxic activities.

2. Results and discussion

Preparation of evodiamine and its analogs has been well documented and is relatively straightforward [12, 13], usually via condensation of 3,4-dihydro-*β*-carboline and

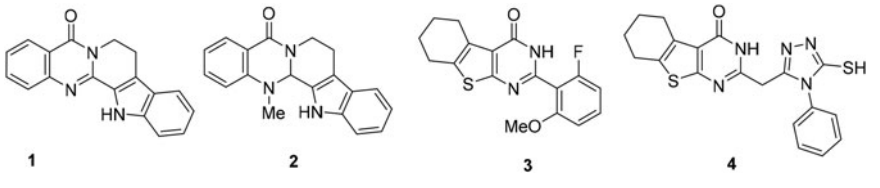
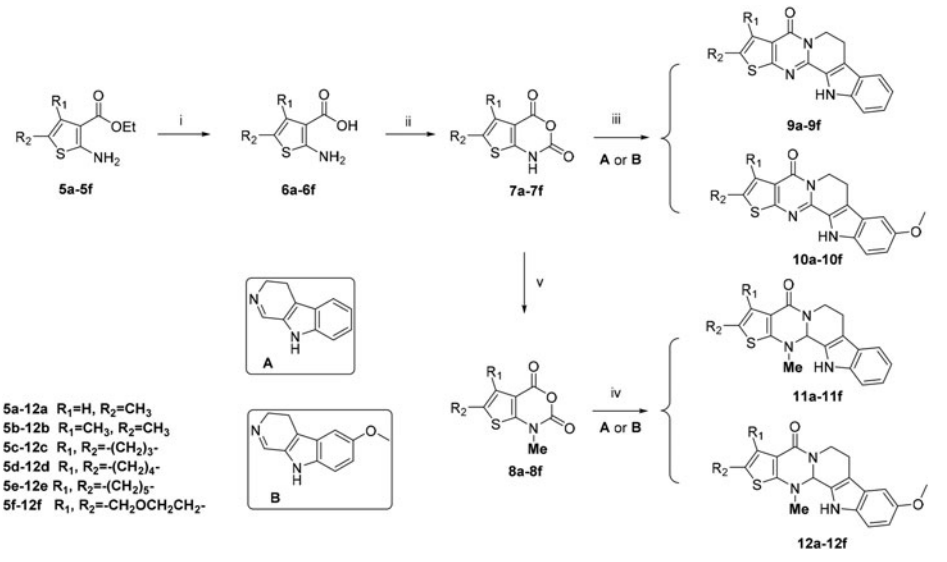


Figure 1. Chemical structure of rutaecarpine (compound **1**), evodiamine (compound **2**), and derivatives of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (compounds **3** and **4**) as potent anticancer reagents.



Scheme 1. Synthesis of hybrids of natural alkaloid evodiamine/rutaecarpine and thieno[2,3-*d*]pyrimidinones. Reagents and conditions: i) Lithium hydroxide (LiOH), ethanol/H_2O, 70 °C, 4 h; ii) triphosgen, 1,4-dioxane, reflux, 20 h, iii) DMF, 95 °C, 24 h; iv) CH_2Cl_2, 95 °C, 24 h; and v) methyl iodide, dimethyl acetamide, diisopropylethylamine, 45 °C overnight.

N-methyl isatoic anhydride. On the other hand, methods for synthesis of rutaecarpine are diversified [6]. We recently developed a one-pot, two-component protocol for fast preparation of rutaecarpine using isatoic anhydride and 3,4-dihydro-*β*-carboline, which is devoid of tedious purification procedures [23]. Considering the structural similarity between isatoic anhydride and 2*H*-thieno[2,3-*d*][1, 3] oxazine-2,4(1*H*)-dione, it is envisioned that this method could also applied for thieno -rutarcarpines.

The synthetic process is elucidated in Scheme 1. Substituted ethyl 2-aminothiophene-3-carboxylates (compound **5a–5f**) were prepared by application of Gewald reaction to cyanoacetate, sulfur and respectful ketone in the presence of trimethylamine. Hydrolysis of esters (compounds **5a–5f)** in hot sodium hydroxide solution (ethanol/water) afforded 2-aminothiophene-3-carboxylic acids (compounds **6a–6f**). Treatment of acids (compounds **6a–6f)** with triphosgene in dry dioxane produced 2*H*-thieno[2,3-*d*][1, 3]oxazine-2,4(1*H*)-diones (compounds **7a–7f**). Condensation of compounds **7a–7f** with 3,4-dihydro-*β*-carboline (compound **A**) or 6-methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (compound **B**) in DMF at 95 °C yielded smoothly the desired hybrids of rutaecarpine and thieno[2,3-*d*]pyrimidinones (compounds **9a–9f** and **10a–10f**).

Methylation of intermediates (compounds **7a–7f**) with iodomethane yielded compounds **8a–8f**, which was then condensed with 3,4-dihydro-*β*-carboline (compound **A** or **B**) in dichloromethane at room temperature, to produce the desired hybrids of evodiamine and thieno[2,3-*d*]pyrimidinones (compounds **11a–11f** and **12a–12f**).

In general, preparation of all the intermediates did not require any chromatographic purification. The target hybrids of rutarcarpines can be collected by filtration of the precipitated solids upon treatment of the reaction mixtures with water. All of

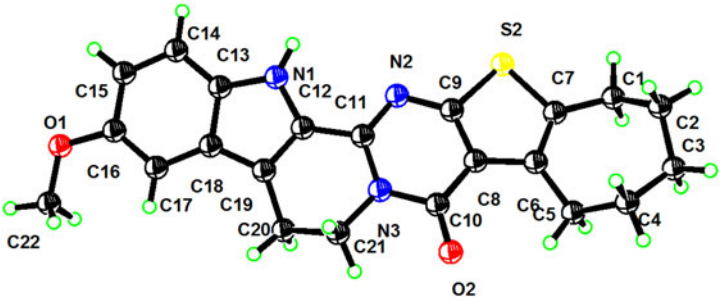


Figure 2. ORTEP representation of compound **11e** (CCDC 1842566).

the targets were purified by column chromatography to ensure the purities for biological assay. The obtained compounds were characterized by melting point, ^{1}H and ^{13}C NMR to confirm their structures. These data are in total agreement with the desired structures. Moreover, the HRMS of the synthesized targets display the correct molecular ion peaks. Compound **11e** was further confirmed by the analysis of X-ray data (Figure 2, CCDC 1842566 contains the supplementary crystallographic data for compound **11e**. The data can be found free of charge at www.ccdc.cam.ac.uk/getstructures).

Considering that series of quinazolinones natural products had been identified as cytotoxic agents to tumor cells [4], we investigated the antiproliferative activity of 24 compounds in several types of human cancer cells including human non-small cell lung cancer cells A549, PC-9, human prostate cancer cells PC-3, and human breast cancer cell line MCF-7. Topoisomerase I inhibitor camptothecin was used as a positive control compound [14]. The result revealed that majority of compounds exhibited moderate cytotoxic activity to all of the A549, PC-9, PC-3, and MCF-7 cells, although compounds **10f** and **12e** were nearly inactive to the four cell lines (Table 1). Compounds **9a**, **10e**, **11a**, **11d**, **11f**, and **12a** exhibited promising antiproliferative activity towards the four cells with individual half maximal inhibitory concentration (IC_{50}) values of around 10 μM.

The structure–activity relationship study revealed that the nature of the substituted group on the thieno[2,3-*d*]pyrimidinone plays an important role in the cytotoxic activity of the compound. In general, the evodiamine hybrids (compounds **11a–11f** (bearing methoxy group at 9-position of indole ring)) exhibit more potent cytotoxicity against four cell lines than rutaecparine hybrids (compounds **9a–9f**), while evodiamine hybrids (compounds **12a–12f** (bearing methoxy group at 9-position of indole ring)) are less potent compared to rutaecparine hybrids (compounds **10a–10f**). Compounds **9d**, **10d**, **12b**, and **12d** displayed better activities to A549, MCF-7 cell lines compared to PC-3 and PC-9 cell lines. Compounds **10f** and **12f** shared similar substitution pattern and they were almost inactive to A549, PC-3, and PC-9 cell lines, although compound **12f** was moderately toxic to MCF-7 cell. These results might indicate those substitution groups were unfavorable for the anti-cancer activity.

Compound **11a** showed the greatest activity to inhibit the proliferation of A549, PC-9, PC-3, and MCF-7 cell lines and there no significant differences among these

Table 1. Cytotoxic activity[a] (IC_{50}, μM) of compounds **9a**–**12f.**

Compound	Cell lines			
	A549	PC-9	MCF-7	PC-3
9a	12.90 ± 3.40	13.30 ± 2.12	12.39 ± 0.81	12.91 ± 0.02
9b	15.65 ± 2.66	12.97 ± 1.65	49.56 ± 1.72	14.91 ± 3.89
9c	15.99 ± 3.49	14.27 ± 4.23	>100	>100
9d	12.14 ± 1.49	74.09 ± 17.13	11.58 ± 1.21	73.04 ± 23.14
9e	8.99 ± 1.39	41.98 ± 11.15	10.47 ± 0.70	14.31 ± 0.36
9f	11.60 ± 1.64	12.34 ± 1.54	50.50 ± 3.45	12.21 ± 0.82
10a	>100	11.44 ± 0.17	89.14 ± 0.22	>100
10b	12.31 ± 0.24	19.43 ± 1.40	9.10 ± 0.10	>100
10c	12.03 ± 2.28	36.07 ± 6.50	11.00 ± 0.59	10.07 ± 0.19
10d	8.35 ± 1.99	83.39 ± 17.51	12.44 ± 1.04	>100
10e	12.15 ± 1.25	13.84 ± 1.82	13.18 ± 1.86	12.60 ± 2.10
10f	>100	59.34 ± 8.06	95.78 ± 2.07	91.86 ± 3.48
11a	10.45 ± 1.27	9.46 ± 0.24	9.02 ± 1.12	7.77 ± 0.77
11b	77.85 ± 6.48	12.87 ± 0.11	12.68 ± 2.81	50.47 ± 8.19
11c	11.52 ± 1.00	10.61 ± 0.31	14.24 ± 2.89	>100
11d	11.20 ± 0.85	12.20 ± 0.81	11.26 ± 1.30	11.45 ± 0.81
11e	17.21 ± 0.65	11.26 ± 0.34	11.66 ± 3.18	41.14 ± 6.07
11f	11.51 ± 0.94	11.60 ± 1.46	10.83 ± 0.65	11.02 ± 0.53
12a	14.70 ± 1.91	9.94 ± 0.33	7.70 ± 1.79	4.21 ± 0.06
12b	12.71 ± 0.92	70.14 ± 6.34	10.72 ± 2.77	48.99 ± 9.96
12c	13.93 ± 1.90	24.75 ± 2.12	12.25 ± 2.60	15.95 ± 4.54
12d	13.16 ± 0.45	73.41 ± 2.50	15.55 ± 3.18	65.89 ± 11.73
12e	66.90 ± 6.75	77.06 ± 10.34	99.29 ± 4.30	47.90 ± 1.30
12f	74.75 ± 6.21	86.53 ± 3.76	14.11 ± 0.66	65.02 ± 15.61
Camptothecin	0.06 ± 0.02	0.15 ± 0.03	0.14 ± 0.04	0.52 ± 0.07

[a]Data are from at least three independent experiments.

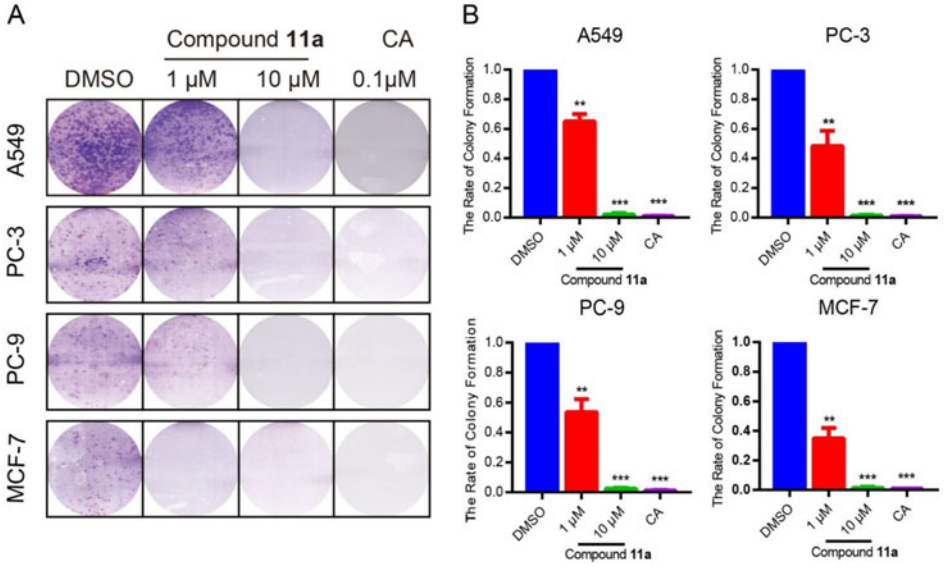


Figure 3. Compound **11a** could inhibit the colony formation of cancer cell lines. (A) The colony formation of A549, PC-9, MCF-7, and PC-3 cells could be reduced by compound **11a** at a dose dependent manner. The compound **11a** incubated with cancer cells for 10 days. (B) Quantification of the rate of colony formation described in A. Statistically significant differences are presented as $^{**}p < 0.01$, $^{***}p < 0.001$, and compared with the control group. CA: camptothecin.

four cancer cells. It may demonstrate that less bulky substation group would be better for the potency, although bulky group was also tolerable in some cases.

Data mentioned above unfolded that compound **11a** might serve as a lead compounds for development of potential anticancer agents. For further evaluation of the active compound, we utilized a colony formation assay to measure inhibition of proliferation on A549, PC-9, PC-3, and MCF-7 cells. Similar to what we observed in a 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, compound **11a** showed strong cell proliferation inhibition activity to the four cell lines (Figure 3). The number of colonies was much lower after incubating with compound **11a** at 10 μM compared to the control groups ($p < 0.001$) in the four cell lines. Based on the results, we found that compound **11a** could dose-dependently inhibit the proliferation of cancer cells. The results indicated that derivatives of quinazolinones possessed potent anti-tumor activities against various types of cancer.

Summarizing, in this research, by taking advantage of our recently reported method of expeditious preparation of rutaecarpines, we synthesized 12 hybrids of natural alkaloid rutaecarpine and thieno[2,3-*d*]pyrimidinones. By condensation of intermediates (compounds **8a–8f)** with 3,4-dihydro-β-carbolines, another 12 hybrids of evodiamine and thieno[2,3-*d*]pyrimidinones were prepared. The synthetic route is straightforward and the targets are easy to be purified. The synthetic compounds displayed moderate to good anticancer activities. The potency of compound **11a** was further confirmed by colony formation assay.

3. Experimental

3.1. General experimental procedures

Reagents and solvents were purchased from commercial suppliers and used without further purification. Thin-layer chromatography was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254, Qingdao, China) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh, Qingdao, China). Melting points were determined on a Buchi B-540 apparatus and uncorrected (Bern, Switzerland). All the NMR spectra were recorded with a Varian 400 or 600 MHz NMR spectrometer, respectively (California, CA, USA) in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO)-d_6, using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were recorded on AB SCIEX QSTAR Elite quadrupole time-of-flight mass spectrometry (California, CA, USA). The detailed synthetic procedure and spectral data of intermediates (compounds **5a-5f**, **6a-6f**, **7a-7f**, **8a-8f**, **A,** and **B)** were described in the supplementary material of this article.

3.2. General procedure of preparation of compounds 9a-9f and 10a-10f

To the solution of thieno[2,3-*d*][1, 3]oxazine-2,4-dione (1 mmol) in DMF (1 ml) was added 3,4-dihydro-β-carboline (compound **A** or **B** (1.3 mmol)). The reaction mixture was heated at 95 °C for 24 hours without protection atmosphere. Then 20 ml of water

were poured into the solution. The precipitated solid was collected by filtration, washed with water, and purified to produce the title compounds.

3.2.1. 2-Methyl-7,12-dihydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(6H)-one (compound 9a)

Yield 76%, light yellow solid, m.p. 278–280 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.14 (s, 1H), 4.56 (t, J = 7.0 Hz, 2H), 3.20 (t, J = 7.0 Hz, 2H), and 2.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 157.8, 144.9, 138.4, 137.3, 127.1, 125.7 (C and CH overlap), 123.4, 120.8, 120.2, 120.2, 118.3, 112.2, 41.1, 19.8, and 16.2. HRTOFMS: m/z 308.0840 $[M+H]^+$ (calcd for $C_{17}H_{13}N_3OS$, 308.0858).

3.2.2. 2,3-Dimethyl-7,12-dihydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(6H)-one (compound 9b)

Yield 82%, light yellow solid, m.p. 310–312 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.5 Hz, 1H), 4.54 (t, J = 7.0 Hz, 2H), 3.20 (t, J = 7.0 Hz, 2H), 2.51 (s, 3H), and 2.39 (s, 3H). HRTOFMS: m/z 322.1002 $[M+H]^+$ (Calcd for $C_{18}H_{15}N_3OS$, 322.1014).

3.2.3. 5,6,9,10,11,14-Hexahydro-8H-cyclopenta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 9c)

Yield 81%, light yellow solid, m.p. 305–307 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.36–7.30 (m, 1H), 7.21–7.15 (m, 1H), 4.55 (t, J = 7.1 Hz, 2H), 3.20 (t, J = 7.1 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.96 (t, J = 7.2 Hz, 2H), and 2.52–2.43(m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 144.4, 141.2, 138.4, 138.3, 127.1, 125.8, 125.7, 120.9, 120.2, 118.7, 118.2, 112.2, 110.17, 40.8 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 28.1(CH_2), and 19.9 (CH_2). HRTOFMS: m/z 334.0987 $[M+H]^+$ (Calcd for $C_{19}H_{15}N_3OS$, 334.1014).

3.2.4. 5,9,10,11,12,15-Hexahydrobenzo[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8(6H)-one (compound 9d)

Yield 88%, light yellow solid, m.p. 326–328 °C; ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD) δ 7.50 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.05 (dd, J = 7.9, 7.0 Hz, 1H), 4.40 (t, J = 7.0 Hz, 2H), 3.08 (t, J = 7.0 Hz, 2H), 2.93 (t, J = 5.8 Hz, 2H), 2.66 (t, J = 5.8 Hz, 2H), and 1.84–1.70 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD) δ 161.4, 158.4, 144.9, 138.6, 133.0, 132.0, 126.5, 125.5 (CH), 125.2, 120.7, 120.4 (CH), 119.9 (CH), 118.3, 112.2 (CH), 40.6, 25.6, 25.2, 22.8, 22.2, and 19.6. HRTOFMS: m/z 348.1157 $[M+H]^+$ (calcd for $C_{20}H_{17}N_3OS$, 348.1171).

3.2.5. 5,6,9,10,11,12,13,16-Octahydro-8H-cyclohepta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 9e)

Yield 91%, light yellow solid, m.p. 275–277 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.34–7.28(m, 1H), 7.20–7.14 (m, 1H), 4.53 (t, J = 7.0 Hz, 2H), 3.4 (t, J = 5.3 Hz, 2H), 3.18 (t, J = 7.0 Hz, 2H), 2.80 (t, J = 5.3 Hz, 2H), 1.96–1.85(m, 2H), and 1.77–1.65(m, 4H). ^{13}C NMR (100 MHz,

$CDCl_3$) δ 160.1, 158.8, 144.3, 138.4, 138.0, 137.3, 127.2, 125.8, 125.6 (CH), 121.7, 120.8 (CH), 120.1 (CH), 118.0, 112.2 (CH), 40.7, 32.8, 30.1, 28.0, 27.9, 27.4, and 19.9. HRTOFMS: *m/z* 362.1314 $[M+H]^+$ (calcd for $C_{21}H_{19}N_3OS$, 362.1327).

3.2.6. 5,6,9,10,12,15-Hexahydro-8H-pyrano[4‴,3‴:4″,5″]thieno[2″,3″:4′,5′]pyrimido[1′,2′:1,2]pyrido[3,4-b]indol-8-one (compound 9f)

Yield 79%, white solid, m.p. 346–348 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 4.76 (s, 2H), 4.40 (t, J = 7.0 Hz, 2H), 3.92 (t, J = 5.4 Hz, 2H), 3.15 (t, J = 7.0 Hz, 2H), and 2.98 (t, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7, 157.2, 145.5, 138.7, 129.3, 128.9, 126.8, 124.8 (C and CH overlapped), 120.0 (CH), 119.8 (CH), 119.6, 117.9, 112.5 (CH), 64.3, 63.9, 54.9, 26.1, and 18.9. HRTOFMS: *m/z* 350.0952 $[M+H]^+$ (calcd for $C_{19}H_{15}N_3O_2S$, 350.0963).

3.2.7. 9-Methoxy-2-methyl-6,7-dihydrothieno[2″,3″:4′,5′]pyrimido[1′,2′:1,2]pyrido[3,4-b]indol-4(12H)-one (compound 10a)

Yield 65%, light yellow solid, m.p.273–275 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 7.29 (t, J = 4.8 Hz, 1H), 7.12 (d, J = 1.1 Hz, 1H), 6.98 (dd, J = 4.8, 2.3 Hz, 2H), 4.55 (t, J = 7.0 Hz, 2H), 3.87 (s, 3H), 3.16 (t, J = 7.0 Hz, 2H), and 2.51 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 157.7, 154.9, 144.9, 137.2, 133.7, 127.6, 126.0, 123.2, 120.2 (CH), 117.8, 116.8 (CH), 113.1 (CH), 100.8 (CH), 55.9 (CH_3), 41.1 (CH_2), 19.9 (CH_2), and 16.2 (CH_3). HRTOFMS: *m/z* 338.0944 $[M+H]^+$ (calcd for $C_{18}H_{15}N_3O_2S$, 338.0963).

3.2.8. 9-Methoxy-2,3-dimethyl-7,12-dihydrothieno[2″,3″:4′,5′]pyrimido[1′,2′:1,2]pyrido[3,4-b]indol-4(6H)-one (compound 10b)

Yield 89%, light yellow solid, m.p. 321–323 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.31 (d, J = 9.7 Hz, 1H), 7.00 (m, 2H), 4.53 (t, J = 7.0 Hz, 2H), 3.87 (s, 3H), 3.16 (t, J = 7.0 Hz, 2H), 2.51 (s, 3H), and 2.39 (s, 3H). HRTOFMS: *m/z* 352.1105 $[M+H]^+$ (calcd for $C_{19}H_{17}N_3OS$, 3521.1120).

3.2.9. 3-Methoxy-9,10,11,14-tetrahydro-5H-cyclopenta[4″,5″]thieno[2″,3″:4′,5′]pyrimido[1′,2′:1,2]pyrido[3,4-b]indol-8(6H)-one (compound 10c)

Yield 83%, light yellow solid, m.p. 308–310 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.30 (d, J = 9.7 Hz, 1H), 7.02–6.94(m, 2H), 4.54 (t, J = 7.0 Hz, 2H), 3.87 (s, 3H), 3.15 (t, J = 7.0 Hz, 2H), 3.09 (t, J = 7.2 Hz, 2H), 2.94 (t, J = 7.2 Hz, 2H), and 2.51–2.41(m, 2H).^{13}C NMR (600 MHz, $CDCl_3$) δ 167.0, 158.3, 154.9, 144.5, 141.2, 138.1, 133.7, 127.8, 126.1, 118.6, 117.6, 116.7 (CH), 113.0 (CH), 100.8 (CH), 58.6 (CH_3), 40.8 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 28.1 (CH_2), and 19.9 (CH_2). HRTOFMS: *m/z* 364.1112$[M+H]^+$ (calcd for $C_{20}H_{17}N_3O_2S$, 364.1120).

3.2.10. 3-Methoxy-5,9,10,11,12,15-hexahydrobenzo[4″,5″]thieno[2″,3″:4′,5′]pyrimido[1′,2′:1,2]pyrido[3,4-b]indol-8(6H)-one (compound 10d)

Yield 87%, light yellow solid, m.p. 274–276 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 7.31 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 6.91 (dd, J = 8.9, 2.4 Hz, 1H), 4.39 (t, J = 7.0 Hz, 2H), 3.79 (s, 3H), 3.12 (t, J = 7.0 Hz, 2H), 2.92

(t, $J = 6.0$ Hz 2H), 2.76 (t, $J = 6.0$ Hz 2H), and 1.87–1.74 (m, 4H). HRTOFMS: *m/z* 362.1313 $[M + H]^+$ (calcd for $C_{21}H_{19}N_3O_2S$, 362.1276).

3.2.11. 3-Methoxy-5,6,9,10,11,12,13,16-octahydro-8H-cyclohepta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 10e)

Yield 95%, white solid, m.p. 270–272 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 7.27–7.21 (m, 1H), 6.99–6.91 (m, 2H), 4.51 (t, $J = 7.0$ Hz, 2H), 3.85 (s, 3H), 3.37 (t, $J = 5.5$ Hz, 2H), 3.13 (t, $J = 7.0$ Hz, 2H), 2.80 (t, $J = 5.5$ Hz, 2H), 1.93–1.83(m, 2H), and 1.74–1.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 158.8, 154.8, 144.4, 137.9, 137.1, 133.7, 127.7, 126.0, 121.6, 117.5, 116.6 (CH), 113.0 (CH), 100.8 (CH), 55.9 (CH_3), 40.7 (CH_2), 32.8 (CH_2), 30.1 (CH_2), 28.0 (CH_2), 27.9 (CH_2), 27.4 (CH_2), and 19.9 (CH_2). HRTOFMS: *m/z* 392.1413 $[M + H]^+$ (calcd for $C_{22}H_{21}N_3O_2S$, 392.1433).

3.2.12. 3-Methoxy-5,6,9,10,12,15-hexahydro-8H-pyrano[4''',3''':4'',5'']thieno[2'',3'':4',5']pyrimido [1',2':1,2]pyrido[3,4-b]indol-8-one (compound 10f)

Yield 82%, white solid, m.p. 306–308 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 7.32 (d, $J = 8.9$ Hz, 1H), 7.11 (d, $J = 2.4$ Hz, 1H), 6.91 (dd, $J = 8.9$, 2.4 Hz, 1H), 4.76 (s, 2H), 4.40 (t, $J = 7.0$ Hz, 2H), 3.92 (t, $J = 5.5$ Hz, 2H), 3.79 (s, 3H), 3.13 (t, $J = 7.0$ Hz, 2H), 2.98 (t, $J = 5.5$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.8, 157.2, 153.8, 145.5, 134.0, 129.1, 128.9, 127.1, 125.0, 119.5, 117.4, 116.1 (CH), 113.3 (CH), 100.5 (CH), 64.3, 63.9, 55.3, 39.1, 26.1, 19.0. HRTOFMS: *m/z* 380.1057 $[M + H]^+$ (calcd for $C_{20}H_{17}N_3O_3S$, 380.1069).

3.3. General procedure of preparation of compounds 11a–11f and 12a–12f

To the solution of 1 mmol of thieno[2,3-*d*][1,3]oxazine-2,4-dione in 3 ml of dichloromethane (CH_2Cl_2), 1.3 mmol of 3,4-dihydro-β-carboline (compound **A** or **B**) was added. The reaction mixture was stirred at room temperature for 24 hours without protection atmosphere. The solvent was evaporated off and the residue was purified to produce the title compounds.

3.3.1. 2,13-Dimethyl-7,12,12b,13-tetrahydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(6H)-one (compound 11a)

Yield 67%, light yellow solid, m.p. 251–253 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.37 (d, $J = 8.1$ Hz, 1H), 7.13 (t, $J = 7.5$ Hz, 1H), 7.03 (t, $J = 7.5$ Hz, 1H), 6.78 (d, $J = 1.1$ Hz, 1H), 6.10 (s, 1H), 4.56 – 4.48 (m, 1H), 3.12 – 3.02 (m, 1H), 2.88 – 2.75 (m, 2H), 2.59 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 159.5, 136.7, 130.0, 128.5, 125.7, 122.0, 121.1, 120.4, 118.9, 118.4, 112.1, 111.6, 70.6, 39.2, 37.8, 19.9, 15.0. HRTOFMS: *m/z* 322.1018 $[M-H]^+$ (calcd for $C_{18}H_{17}N_3OS$, 322.1014).

3.3.2. 2,3,13-Trimethyl-7,12,12b,13-tetrahydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(6H)-one (compound 11b)

Yield 82%, light yellow solid, m.p. 229–331 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.52 (d, $J = 7.8$ Hz, 1H), 7.37 (d, $J = 8.1$ Hz, 1H), 7.13 (t, $J = 7.5$ Hz,

1H), 7.03 (t, $J = 7.5$ Hz, 1H), 6.04 (s, 1H), 4.57–4.49(m, 1H), 3.12–3.03(m, 1H), 2.89–2.72(m, 2H), 2.57 (s, 3H), 2.26 (s, 3H), and 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.1, 158.4, 136.7, 130.2, 128.5, 125.7, 122.6, 122.0, 119.0, 118.9, 118.4, 112.1, 111.6, 70.1, 70.6, 39.2, 37.8, 19.9, 15.0, 13.0, and 12.2. HRTOFMS: *m/z* 336.1177 $[M\text{-}H]^+$ (calcd for $C_{19}H_{19}N_3OS$, 336.1093).

3.3.3 13-Methyl-5,6,9,10,11,13,13a,14-octahydro-8H-cyclopenta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 11c)

Yield 78%, white solid, m.p. 283–285 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.36 (d, $J = 8.1$ Hz, 1H), 7.13 (t, $J = 7.4$ Hz, 1H), 7.03 (t, $J = 7.4$ Hz, 1H), 6.13 (s, 1H), 4.56–4.48(m, 1H), 3.12– 3.05 (m, 1H), 2.90–2.82 (m, 2H), 2.82–2.70(m, 4H), 2.60 (s, 3H), 2.35–2.28 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.1, 161.8, 141.5, 136.7, 130.3, 128.5, 125.7, 122.0 (CH), 118.9 (CH), 118.4 (CH), 115.8, 112.1, 111.6 (CH), 70.8, 39.7, 37.8, 28.9, 28.5, 27.3, and 19.9. HRTOFMS: *m/z* 348.1164 $[M + H]^+$ (calcd for $C_{20}H_{19}N_3OS$, 348.1171).

3.3.4. 14-Methyl-5,6,9,10,11,12,14,14a-octahydrobenzo[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2] pyrido[3,4-b]indol-8(15H)-one (compound 11d)

Yield 93%, light yellow solid, m.p. 321–323 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.51 (d, $J = 7.9$ Hz, 1H), 7.36 (d, $J = 8.1$ Hz, 1H), 7.13 (t, $J = 7.6$ Hz, 1H), 7.02 (t, $J = 7.4$ Hz, 1H), 6.06 (s, 1H), 4.51 (ddd, $J = 12.9$, 5.1, 2.1 Hz, 1H), 3.07 (ddd, $J = 12.6$, 10.8, 4.6 Hz, 1H), 2.97–2.72 (m, 3H), 2.67–2.57 (m, 5H), and 1.80–1.62 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 159.1, 136.7, 132.4, 128.6, 125.7, 125.6, 122.0, 118.9, 118.4, 117.8, 112.1, 111.6, 70.3, 39.0, 37.7, 25.4, 24.2, 22.8, and 22.0 19.9. HRTOFMS: *m/z* 362.1317 $[M\text{-}H]^+$ (calcd for $C_{21}H_{21}N_3OS$, 362.1327).

3.3.5. 15-Methyl-9,10,11,12,13,15,15a,16-octahydro-5H-cyclohepta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8(6H)-one (compound 11e)

Yield 95%, light yellow solid, m.p. 321–323 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H), 7.50 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.15 – 7.06 (t, $J = 7.4$ Hz, 1H), 7.00 (t, $J = 7.4$ Hz, 1H), 6.00 (s, 1H), 4.55 – 4.46 (m, 1H), 3.21–3.12 (m, 1H), 3.10–2.96(m, 2H), 2.84 (d, $J = 15.1$ Hz, 1H), 2.79–2.71(m, 1H), 2.70–2.62(m, 2H), 2.50 (s, 3H), 1.84–1.73(m, 2H), and 1.61–1.47(m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ δ 162.1, 157.5, 137.9, 136.7, 129.8, 128.4, 125.6, 122.0, 119.5, 118.9, 118.5, 112.14, 111.6, 69.9, 38.8, 37.6, 32.0, 28.7, 27.6, 27.3, 26.9, and 19.9. HRTOFMS: *m/z* 378.1269 $[M + H]^+$ (calcd for $C_{22}H_{23}N_3OS$, 378.1640).

3.3.6. 14-Methyl-5,6,9,10,12,14,14a,15-octahydro-8H-pyrano[4''',3''':4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 11f)

Yield 86%, white solid, m.p. 300–302 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.37 (d, $J = 8.1$ Hz, 1H), 7.16–7.11 (m, 1H), 7.06–7.01 (m, 1H), 6.11 (s, 1H), 4.66–4.57(m, 2H), 4.55–4.49(m, 1H), 3.87–3.80 (m, 2H), 3.11–3.05 (m, 1H), 3.01–2.93 (m, 1H), 2.87–2.76 (m, 2H), 2.77–2.68 (m, 1H), and 2.65 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.0, 160.0, 136.7, 130.1, 128.6, 125.7, 123.0, 122.1 (CH), 118.9 (CH), 118.45 (CH), 117.0, 112.2, 111.6 (CH), 70.5,

64.1, 64.0, 39.1, 37.9, 26.1, and 19.8. HRTOFMS: *m/z* 366.1257 $[M+H]^+$ (calcd for $C_{20}H_{19}N_3O_2S$, 366.1276).

3.3.7. 2,13-Dimethyl-6,7,12b,13-tetrahydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(12H)-one (compound 12a)

Yield 72%, light yellow solid, m.p. 321–323 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.26 (d, $J=8.8$ Hz, 1H), 7.01 (d, $J=2.4$ Hz, 1H), 6.80–6.75 (m, 2H), 6.07 (s, 1H), 4.56–4.47(m, 1H), 3.76 (s, 3H), 3.06 (td, $J=11.9$, 4.5 Hz, 1H), 2.86–2.72 (m, 2H), 2.59 (s, 3H), and 2.36 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 159.4, 153.4, 131.7, 129.9, 129.0, 126.0, 121.2, 121.1, 120.3, 112.3, 112.2, 112.2, 111.9, 100.2, 70.7, 55.4, 39.3, 37.7, 20.0, and 15.0. HRTOFMS: *m/z* 352.1124 $[M+H]^+$ (calcd for $C_{19}H_{19}N_3OS$, 352.1120).

3.3.8. 3-Methoxy-14-methyl-5,6,9,10,12,14,14a,15-octahydro-9-methoxy-2,3,13-trimethyl-7,12,12b,13-tetrahydrothieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-4(6H)-one (compound 12b)

Yield 79%, light yellow solid, m.p. 213–215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.25 (d, $J=8.8$ Hz, 1H), 7.01 (d, $J=2.2$ Hz, 1H), 6.77 (dd, $J=8.8$, 2.2 Hz, 1H), 6.00 (s, 1H), 4.57– 4.47 (m, 1H), 3.76 (s, 3H), 3.11–3.01 (m, 1H), 2.86–2.71 (m, 2H), 2.56 (s, 3H), 2.25 (s, 3H), and 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 158.4, 153.4, 131.7, 130.2, 129.1, 125.9, 122.4, 118.8, 112.3, 112.2, 111.9, 100.21, 70.2, 55.3, 38.9, 37.5, 120.0, 13.0, and 12.1. HRTOFMS: *m/z* 366.1272 $[M-H]^+$ (calcd for $C_{20}H_{21}N_3O_2S$, 366.1276).

3.3.9. 3-Methoxy-13-methyl-5,6,9,10,11,13,13a,14-octahydro-8H-cyclopenta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2] pyrido[3,4-b]indol-8-one (compound 12c)

Yield 85%, light yellow solid, m.p. 205–207 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.25 (d, $J=8.8$ Hz, 1H), 7.01 (d, $J=1.6$ Hz, 1H), 6.90 – 6.74 (m, 1H), 6.10 (s, 1H), 4.54–4.47 (m, 1H), 3.76 (s, 3H), 3.10–3.01(m, 1H), 2.99–2.69 (m, 6H), 2.60 (s, 3Hand 2.35–2.27 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 161.8, 153.4, 141.5, 131.7, 130.2, 129.1, 126.0, 115.7, 112.3 (CH), 112.2 (CH), 111.9, 100.2 (CH), 70.8, 55.4, 39.1, 37.8, 28.9, 28.5, 27.34, and 19.94. HRTOFMS: *m/z* 380.1421 $[M+H]^+$ (calcd for $C_{21}H_{21}N_3O_2S$, 380.1433).

3.3.10. 3-Methoxy-14-methyl-5,6,9,10,11,12,14,14a-octahydrobenzo[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8(15H)-one (compound 12d)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.25 (d, $J=8.8$ Hz, 1H), 7.00 (d, $J=2.4$ Hz, 1H), 6.77 (dd, $J=8.8$, 2.4 Hz, 1H), 6.03 (s, 1H), 4.54–4.46(m, 1H), 3.76 (s, 3H), 3.05 (ddd, $J=12.6$, 10.9, 4.6 Hz, 1H), 2.96–2.85(m, 1H), 2.86–2.68 (m, 2H), 2.67–2.56 (m, 2H), 2.59 (s, 3H), and 1.80–1.63 (m, 4H). ^{13}C NMR (101 MHz, dmso) δ 162.1, 159.1, 153.3, 132.4 131.7, 129.2, 126.0, 125.5, 117.7, 112.3, 112.18, 111.9, 100.2, 70.4, 55.4, 39.0, 37.7, 25.4, 24.2, 22.8, 22.0, and 20.0. HRTOFMS: *m/z* 392.1421 $[M-H]^+$ (calcd for $C_{22}H_{23}N_3O_2S$, 392.1433).

3.3.11. 3-Methoxy-15-methyl-9,10,11,12,13,15,15a,16-octahydro-5H-cyclohepta[4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8(6H)-one (compound 12e)

Yield 88%, light yellow solid, m.p. 321–323 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.25 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 2.4 Hz, 1H), 6.77 (dd, J = 8.8, 2.4 Hz, 1H), 6.00 (d, J = 1.5 Hz, 1H), 4.56–4.47 (m, 1H), 3.76 (s, 2H), 3.22–3.12 (m, 1H), 3.11–2.97 (m, 2H), 2.87–2.80 (m, 1H), 2.79–2.65(m, 3H), 2.53 (s, 3H), 1.84–1.77(m, 2H), and 1.64–1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 157.5, 153.4, 137.9, 131.8, 129.6, 129.0, 125.9, 119.3, 112.3, 112.2, 111.9, 100.2, 69.9, 55.4, 39.5, 38.9, 37.5, 32.0, 28.7, 27.6, 27.3, 26.9, and 20.0. HRTOFMS: m/z 406.1764 $[M\text{-}H]^+$ (calcd for $C_{23}H_{25}N_3O_2S$, 406.1589).

3.3.12. 3-Methoxy-14-methyl-5,6,9,10,12,14,14a,15-octahydro-8H-pyrano[4''',3''':4'',5'']thieno[2'',3'':4',5']pyrimido[1',2':1,2]pyrido[3,4-b]indol-8-one (compound 12f)

Yield 88%, white solid, m.p. 262–264 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.26 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 2.3 Hz, 1H), 6.78 (dd, J = 8.8, 2.3 Hz, 1H), 6.08 (s, 1H), 4.65–4.57(m, 2H), 4.54–4.49 (m, 1H), 3.87–3.80 (m, 2H), 3.76 (s, 3H), 3.09–3.0.3(m, 1H), 3.01–2.94 (m, 1H), 2.83–2.75(m, 2H), 2.73– 2.67(m, 1H), and 2.65 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.0, 160.0, 153.4, 131.7, 130.1, 129.1, 126.0, 122.9, 116.9, 112.3, 112.2, 112.0, 100.2, 70.6, 64.1, 64.0, 55.4, 39.9, 37.9, 26.2, and 19.9. HRTOFMS: m/z 396.1361 $[M + H]^+$ (calcd for $C_{21}H_{21}N_3O_3S$, 396.1382).

3.4. Biological activity

3.4.1. Cell culture

All human cell lines were mycoplasma-tested and obtained from the American Type Culture Collection. The human non-small cell lung cancer (NSCLC) cell lines A549 and PC-9 were cultured in Roswell Park Memorial Institute medium (RPMI) 1640 medium while the human breast cancer cell line MCF-7 were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin). The human prostate cancer cell line PC-3 cells were incubated with Ham's F-12 medium supplemented with 10% FBS, 1% penicillin/streptomycin and 1% glutamine. All cells were cultured at 37 °C in a humidified 5% carbon dioxide atmosphere.

3.4.2. MTT assay

Cells were seeded into 96-well plates (4×10^3 cells/well) and cultured in medium for 24h respectively and then exposed to 24 chemotherapeutic agents with various concentrations (100, 10, 1, 0.1, and 0.01 μM). Besides, camptothecin and DMSO served as a positive control and a negative control, respectively. After 72h of incubation, MTT solution (5 mg/ml in sterilized phosphate-buffer saline; PBS) of 20 μl/well was added into each well and incubated for another four hours. Sequentially, 100 μl of lysis solution (10% sodium dodecyl sulfate- 5% isobutyl alcohol-0.01M HCl) was added into per well in order to dissolve the formazan crystals. After dissolving overnight, the optical density (OD) was measured using a microplate reader at 570 nm. The inhibitory rates of these compounds were calculated by the following formula: $(OD_{control} - OD_{treated})/OD_{control} \times 100\%$. The activity of compounds were evaluated by IC_{50} values.

3.4.3. Colony formation assays

Briefly, after being washed, trypsinized and counted, cells (4000 cells/well) were seeded in six-well plates and cultured in medium overnight. After being treated with drugs (1 and 10 μM) and camptothecin (0.1 μM) for 10 days, the supernatant of per well was discarded and fresh PBS (1 ml/well) was added to wash the cells twice. Subsequently, 1 ml of staining solution (10% acetic acid- 10% methanol- 80% purified water) was added to fix the cells at room temperature for 30 min. Sequentially, the staining solution was poured out and washed with running water. Then, 1% crystal violet staining solution (dissolved in methanol) was added to per well and co-incubated with cells at room temperature for 30 min. The number of colonies purple was counted for cell viability.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by 12th Chinese Recruitment Program of Global Experts (Thousand Talents Program) and Research Program of Shanghai Normal Univeristy (DYL201702).

ORCID

Haji Akber Aisa http://orcid.org/0000-0003-4652-6879
Guo-Zheng Huang http://orcid.org/0000-0002-5730-9549

References

[1] M. Asif, *Int. J. Med. Chem*. **2014**, 395637 (2014).
[2] E. Jafari, M.R. Khajouei, F. Hassanzadeh, G.H. Hakimelahi, and G.A. Khodarahmi, *Res. Pharm. Sci*. **11**, 1 (2016).
[3] I. Khan, A. Ibrar, N. Abbas, and A. Saeed, *Eur. J. Med. Chem*.**76**, 193 (2014).
[4] I. Khan, S. Zaib, S. Batool, N. Abbas, Z. Ashraf, J. Iqbal, and A. Saeed, *Bioorg. Med. Chem*. **24**, 2361 (2016).
[5] J.P. Michael, *Nat. Prod. Rep*. **22**, 627 (2005).
[6] J.K. Son, H.W. Chang, Y. Jahng, *Molecules*. **20**, 10800 (2015).
[7]] G. Poli, J.P. Dai, W.Z. Li, X.F. Zhao, G.F. Wang, J.C. Yang, L. Zhang, X.X. Chen, Y.X. Xu, and K.S. Li, *PLoS ONE*. **7**, e42706 (2012).
[8] S.M. Yuan, K. Gao, D.M. Wang, X.Z. Quan, J.N. Liu, C.M. Ma, C. Qin, and L.F. Zhang, *Acta Pharmacol. Sin*. **32**, 295 (2011).
[9] G. Huang, B. Kling, F.H. Darras, J. Heilmann, and M. Decker, *Eur. J. Med. Chem*. **81**, 15 (2014).
[10] J. Jiang and C. Hu, *Molecules* **14**, 1852 (2009).
[11] Z. Chen, G. Hu, D. Li, J. Chen, Y. Li, H. Zhou, and Y. Xie, *Bioorg. Med. Chem*. **17**, 2351 (2009).
[12] G. Dong, S. Wang, Z. Miao, J. Yao, Y. Zhang, Z. Guo, W. Zhang, and C. Sheng, *J. Med. Chem*. **55**, 7593 (2012).

[13] S. Wang, K. Fang, G. Dong, S. Chen, N. Liu, Z. Miao, J. Yao, J. Li, W. Zhang, and C. Sheng, *J. Med. Chem*. **58**, 6678 (2015).
[14] D D.B. Khadka and W.J. Cho, *Exp. Opin. Ther. Pat*. **23**, 1033 (2013).
[15] X.Q. Nie, H.H. Chen, J.Y. Zhang, Y.J. Zhang, J.W. Yang, H.J. Pan, W.X. Song, F. Murad, Y.Q. He, and K. Bian, *Acta Pharmacol. Sin*. **37**, 483 (2016).
[16] S.W. Jin, Y.P. Hwang, C.Y. Choi, H.G. Kim, S.J. Kim, Y. Kim, Y.C. Chung, K.J. Lee, T.C. Jeong, and H.G. Jeong, *Food Chem. Toxicol*. **100**, 138 (2017).
[17] S. He, G. Dong, Z. Wang, W. Chen, Y. Huang, Z. Li, Y. Jiang, N. Liu, J. Yao, Z. Miao, W. Zhang, and C. Sheng, *ACS Med. Chem. Lett*. **6**, 239 (2015).
[18] X. Hu, Y. Wang, J. Xue, T. Han, R. Jiao, Z. Li, W. Liu, F. Xu, H. Hua, and D. Li, *Bioorg. Med. Chem. Lett*. **27**, 4989 (2017).
[19] C. Viegas-Junior, A. Danuello, V. da Silva Bolzani, E. J. Barreiro, and C. A. M. Fraga, *Curr. Med. Chem*. ***14***, 1829 (2007).
[20] Y.D. Wang, S. Johnson, D. Powell, J.P. McGinnis, M. Miranda, and S.K. Rabindran, *Bioorg. Med. Chem. Lett*.**15**, 3763 (2005).
[21] A.T. Mavrova, D. Wesselinova, J.A. Tsenov, and L.A. Lubenov, *Eur. J. Med. Chem*. **86**, 676 (2014).
[22] G. Huang, A. Drakopoulos, M. Saedtler, H. Zou, L. Meinel, J. Heilmann, and M. Decker, *Bioorg. Med. Chem. Lett*. **27**, 4937 (2017).
[23] G. Huang, D. Roos, P. Stadtmüller, and M. Decker, *Tetrahedron Lett*. **55**, 3607 (2014).