# SYNTHESIS AND ANTITUMOR ACTIVITY OF NOVEL LINEAR TRICYCLIC COMPOUNDS DERIVED FROM PURINE

**Hongmei Guo,[1,2] Lifei Nie,[1] Khurshed Bozorov,[1,3] Haji Akber Aisa,[1,2]\* and Jiangyu Zhao[1,2]\***

[1] 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; \*E mail: haji@ms.xjb.ac.cn

[2] University of Chinese Academy of Sciences, Beijing, 100049, China

[3] Faculty of Chemistry, Samarkand State University, Samarkand, Uzbekistan

**Abstract** – Synthesis and antitumor activity of two series of novel linear tricyclic purine derivatives were reported. The structures of new compounds were characterized by ^{1}H NMR, ^{13}C NMR, HRMS (ESI). The inhibitory activities of the synthesized compounds were evaluated by conventional MTT assay against HT-29 human colorectal adenocarcinoma cell line, MCF-7 breast cancer cell line, and HeLa human cervical cancer cell. The compounds **5n~5q** with long-chain alkane revealed a better inhibitory effect against all three kinds of cancer cells, while the compounds **4a~4l** and **5a~5l** with different substituted phenyl seemed to be more beneficial for the HeLa cell. Meanwhile, the toxicity of compounds **5m~5q** against L-02 normal human liver cell was tested. The compound **5q** showed promising antitumor activity *in vitro* against HT-29 (IC$_{50}$ = 3.28 ± 0.57 μM), MCF-7 (IC$_{50}$ = 7.13 ± 0.27 μM), and HeLa (IC$_{50}$ = 14.24 ± 0.41 μM) cell lines and relatively low toxicity against L-02 (IC$_{50}$ >100 μM) cell. The results suggested that compound **5q** could serve as a promising antitumor lead compound for further research.

## INTRODUCTION

International Agency for Research on Cancer published GLOBOCAN 2020 estimated the latest cancer incidence and mortality and pointed out 19.3 million new cancer cases and nearly 10 million cancer deaths every year worldwide.[1] Cancer, featuring uncontrolled growth of abnormal cells and sometimes metastasis, remains one of the leading causes of death globally.[2] However, limited by side effects and

drug resistance, it is critically important to find newer and safer anticancer drugs due to current therapy.[3,4] Among innumerable bioactive N-heterocycles molecules, compounds with purine-scaffold have become an interesting fragment for researchers to find promising lead compounds in medicinal chemistry.[5-8] Various medicines with purine core have been widely applied to treat antiviral (acyclovir), anti-inflammatory (azathioprine), antiplatelet (cangrelor tetrasodium), and antibacterial effect (puromycin dihydrochloride), especially antitumor (6-mercaptopurine, duvelisib, nelarabine, clofarabine) (Figure 1). Therefore, it is still very attractive to researchers to design and synthesize new novel derivatives based on purine core.[9-11] Most of bioactive purines and their analogs are obtained by attaching various substituents to the N-1, C-2, N-3, C-6, N-7, C-8, and N-9 positions of the purine skeleton.[12-14] Besides, heterocycle-fused purines are also the hotspots in drug research and development.[15]
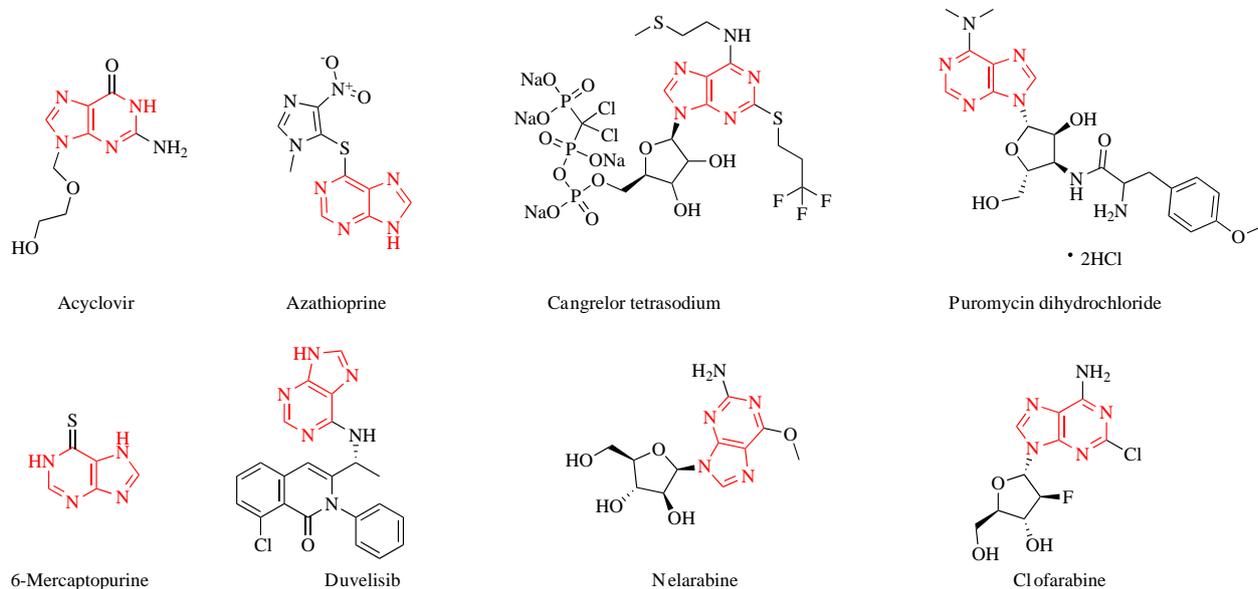


**Figure 1.** Drugs with purine core

Over the past decade, our group had dedicated to the design and structural modification of linear N-heterotricyclic compounds from natural product mackinazolinone and its analogues to find promising anti-tumor lead compounds.[16] And an imidazole precursor was found to exhibit excellent antitumor activity.[17] As shown in Figure 2, a novel series of linear tricyclic purines derivative were designed by introducing an imidazole ring based on bioisosterism. Herein, we reported the synthesis of two series of novel tricyclic compounds derived from purine and preliminary anticancer activities *in vitro*.
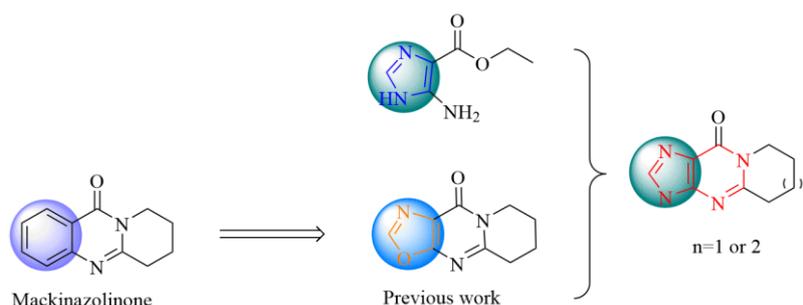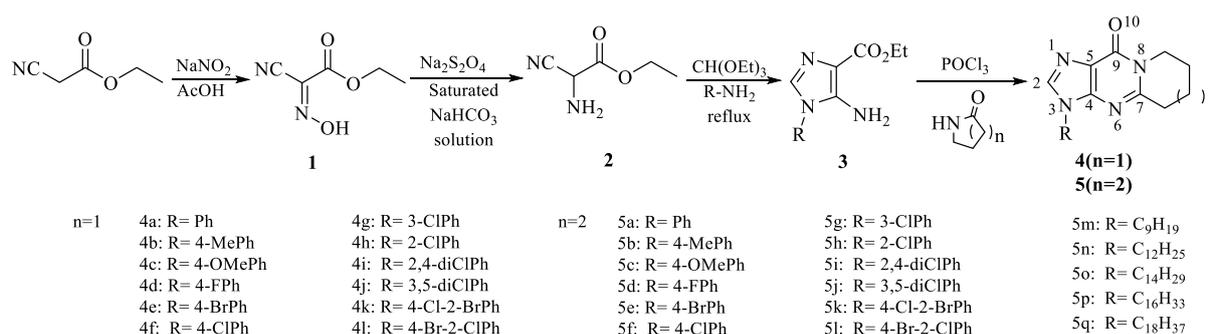
**Figure 2.** Design of novel linear tricyclic purine derivatives

## RESULTS AND DISCUSSION

**Synthesis.** The synthetic route of the target compounds **4a~4l** and **5a~5q** was shown in Scheme 1. Firstly, ethyl cyanohydroxyiminoacetate **1** is obtained by using ethyl cyanoacetate and sodium nitrite as the starting materials in the presence of acetic acid. Then, intermediate **2** was obtained by reduction with sodium hydrosulfite in saturated sodium bicarbonate solution.[18] Subsequently, treating intermediate **2** with proper amines and triethyl orthoformate gave imidazole compounds **3a~3q**.[17] Finally, the reactions between imidazole compounds **3a~3q** and appropriate lactams afforded the condensation compounds **4a~4l** and **5a~5q** in the presence of phosphorus oxychloride.[19] All the structures of the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR spectroscopy, and HRMS analysis. In particular, the structure of compound **4f** (CCDC Deposition No. 2133010) was further verified by X-ray diffraction (XRD) analysis (Figure 3).



| n=1 | 4a: R= Ph | 4g: R= 3-ClPh | n=2 | 5a: R= Ph | 5g: R= 3-ClPh | 5m: R= $C_9H_{19}$ |
| | 4b: R= 4-MePh | 4h: R= 2-ClPh | | 5b: R= 4-MePh | 5h: R= 2-ClPh | 5n: R= $C_{12}H_{25}$ |
| | 4c: R= 4-OMePh | 4i: R= 2,4-diClPh | | 5c: R= 4-OMePh | 5i: R= 2,4-diClPh | 5o: R= $C_{14}H_{29}$ |
| | 4d: R= 4-FPh | 4j: R= 3,5-diClPh | | 5d: R= 4-FPh | 5j: R= 3,5-diClPh | 5p: R= $C_{16}H_{33}$ |
| | 4e: R= 4-BrPh | 4k: R= 4-Cl-2-BrPh | | 5e: R= 4-BrPh | 5k: R= 4-Cl-2-BrPh | 5q: R= $C_{18}H_{37}$ |
| | 4f: R= 4-ClPh | 4l: R= 4-Br-2-ClPh | | 5f: R= 4-ClPh | 5l: R= 4-Br-2-ClPh | |

**Scheme 1.** General synthetic route for the preparation of linear tricyclic purine derivatives **4a~4l** and **5a~5q**
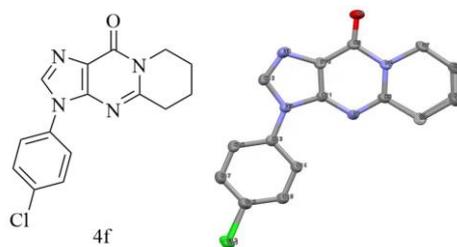
**Figure 3.** Crystal structure of **4f** (CCDC Deposition No. 2133010)

**Antitumor activity.** The *in vitro* antitumor activities of the synthesized compounds were evaluated by conventional MTT assay against HT-29 human colorectal adenocarcinoma cell line, MCF-7 breast cancer cell line, and HeLa human cervical cancer cell with doxycycline (DOX) as a positive control. Compounds **5m~5q** with relatively better activity were selected for toxicity test on L-02 normal cell. The results (Table 1) indicated that most of the compounds showed satisfactory inhibitory activity against the selected cell lines at 50 μM. In the aspect of the structure-activity relationship (SAR), it was found that the substitution of R groups at position N-3 displayed a noticeable impact on the antitumor activity.

**Table 1.** Cell inhibitory activity of synthesized compounds on selected cell lines *in vitro* [a]

| Compd. | IC$_{50}$ (μM) | | | |
|:---:|:---:|:---:|:---:|:---:|
| | HT-29 | MCF-7 | HeLa | L-02 |
| **4a** | -[b] | -[b] | 20.74 ± 0.85 | -[c] |
| **4b** | -[b] | -[b] | 51.85 ± 2.69 | -[c] |
| **4c** | -[b] | -[b] | -[b] | -[c] |
| **4d** | -[b] | -[b] | -[b] | -[c] |
| **4e** | -[b] | -[b] | 14.47 ± 0.69 | -[c] |
| **4f** | -[b] | -[b] | 23.26 ± 4.24 | -[c] |
| **4g** | -[b] | -[b] | -[b] | -[c] |
| **4h** | -[b] | -[b] | -[b] | -[c] |
| **4i** | -[b] | -[b] | 12.32 ± 0.54 | -[c] |
| **4j** | -[b] | -[b] | 52.25 ± 2.77 | -[c] |
| **4k** | -[b] | -[b] | 42.55 ± 1.22 | -[c] |
| **4l** | -[b] | -[b] | 28.99 ± 1.41 | -[c] |
| **5a** | -[b] | -[b] | -[b] | -[c] |
| **5b** | -[b] | -[b] | 21.17 ± 0.97 | -[c] |
| **5c** | -[b] | -[b] | -[b] | -[c] |
| **5d** | -[b] | -[b] | -[b] | -[c] |
| **5e** | -[b] | -[b] | 10.10 ± 0.56 | -[c] |
| **5f** | -[b] | -[b] | 7.50 ± 0.28 | -[c] |
| **5g** | -[b] | -[b] | -[b] | -[c] |
| **5h** | -[b] | -[b] | -[b] | -[c] |
| **5i** | -[b] | -[b] | 17.14 ± 0.78 | -[c] |
| **5j** | -[b] | -[b] | 21.17 ± 0.97 | -[c] |
| **5k** | -[b] | -[b] | -[b] | -[c] |
| **5l** | -[b] | -[b] | -[b] | -[c] |

| | | | |
|---|---|---|---|
| **5m** | 44.32 ± 5.07 | -[b] | -[b] | 62.21 ± 10.65 |
| **5n** | 15.01 ± 1.09 | 41.92 ± 7.37 | 17.95 ± 2.00 | 32.97±1.32 |
| **5o** | 12.55 ± 0.19 | 22.98 ± 2.81 | 19.77 ± 3.73 | 16.83±0.89 |
| **5p** | 9.62 ± 1.82 | 22.28 ± 0.62 | 19.21 ± 1.48 | 28.75±0.67 |
| **5q** | 3.28 ± 0.57 | 7.13 ± 0.27 | 14.24 ± 0.41 | > 100 |
| **DOX** | 0.35 ± 0.02 | 0.94 ± 0.09 | 0.30 ± 0.01 | -[c] |

[a]: the values are mean ± SD% of three replicates
[b]: the inhibition rates of the measured compounds are lower than 50%
[c]: Not tested

On the one hand, when R was different substituted phenyl, compounds had better inhibitory activity against the HeLa cell line than HT-29 and MCF-7 cell lines. However, the results of the two series of compounds **4a~4l** and **5a~5q** were slightly different. By comparing **4a** (20.74 ± 0.85 μM), **4b** (51.85 ± 2.69 μM), and **4c**, it could be evidently observed that the inhibitory effect of the compounds became worse with the enhancement of the electron-donating ability of the substituent. When the *para* position was replaced by a halogen atom, the sequence is Br (**4d**, 14.47 ± 0.69 μM) > Cl (**4f**, 23.26 ± 4.24 μM) > F (**4e**). Moreover, it was found that the effect of the substituent in the *para* position was better, by comparing the *para* (**4f**), *meta* (**4g**), and *ortho* (**4h**) positions of chlorine atoms. It was surprising that 2,4-dichlorophenyl substituted compound **4i** (12.32 ± 0.54 μM) showed better activity than the other di-halogen-substituted compound **4j** (52.25 ± 2.77 μM), **4k** (42.55 ± 1.22 μM), **4l** (28.99 ± 1.41 μM).

As for compounds **5a**, **5b**, and **5c**, only **5b** showed moderate activity (21.17 ± 0.97 μM). Similarly, we found that the introduction of the F atom did not improve the activity by comparing different halogen-substituted compounds **5d** (F), **5e** (Br, 10.10 ± 0.56 μM), and **5f** (Cl, 7.50 ± 0.28 μM). In addition, 2,4-dichlorophenyl substituted compound **5i** still showed the best activity among the di-halogen-substituted compounds (**5j**, 21.17 ± 0.97 μM, **5k**, **5l**).

On the other hand, when R was a long-chain alkyl, compounds exhibited promising inhibitory activity on all three kinds of cancer cells, except for compound **5m**, which was only potent on the HT-29 cell line. Moreover, it was worth mentioning that the length of the carbon chain has a great effect on the activity. For example, when the number of C atoms increased from 12 to 18, the $IC_{50}$ of the compounds against the HT-29 cell line decreased greatly from 44.32 ± 5.07 μM to 3.28 ± 0.57 μM. A similar trend was also observed against MCF-7 and HeLa cell lines. However, for normal cells, there was no clear relationship between the length of the carbon chain and the growth inhibition against L-02 normal cells. By comparison, it could be found that the compounds **5m~5q** could selectively inhibit the growth of cancer cells. And it was encouraging that compound **5q** showed the most promising anticancer activity than others on all cells (3.28 ± 0.57 μM for HT-29, 7.13 ± 0.27 μM for MCF-7, 14.24 ± 0.41 μM for HeLa), and the lowest toxicity against L-02 cell (>100 μM). Based on the above SAR, the antitumor activity of

compounds **5a~5q** with a seven-membered ring (n = 2) was superior to that of six-membered rings compounds **4a~4l** (n = 1).

## CONCLUSION

In summary, twenty-nine novel linear tricyclic purine derivatives were designed and synthesized. And the inhibitory activities against HT-29, MCF-7, and HeLa cell lines were evaluated *in vitro*. Most of them displayed promising antitumor activities against three cell lines. The SAR study indicated that chlorine and bromine atoms in the phenyl group could markedly improve the inhibition activity. The introduction of long-chain alkane at the N-3 position of the synthesized purine derivatives was more favorable for antitumor activity. Compound **5q** was the most potent against the selected tumor cell lines and lowest toxic against L-02 cell line, so it was worthy of further study as the antitumor lead compound.

## EXPERIMENTAL

**General methods.** All reagents and solvents (analytical grade) are purchased from commercial suppliers (Tansoole.com) and used directly without further purification. The reaction process was monitored by silica gel thin layer chromatography (TLC) (GF-254; Qingdao Haiyang Chemical Co.), visualized under UV light (254 nm) (ZF-20D black box ultraviolet analyzer). The products were purified by column chromatography over silica gel (100-200 mesh, Qingdao Haiyang Chemical Co.). Melting points were determined on a Buchi B-540 apparatus and were uncorrected. $^1$H and $^{13}$C NMR spectra were achieved with a VARIAN MR 400 and BRUKER AVANCE NEO 600 spectrometer with tetramethylsilane (TMS) as an internal standard (600 and 400 MHz for $^1$H, 150 and 100 MHz for $^{13}$C). $^1$H and $^{13}$C chemical shifts were reported in parts per million (ppm, d). 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 compound 1.** A mixture of ethyl cyanoacetate (11.3 g, 0.1 mol) and 45% acetic acid aqueous solution (45 mL) was stirred at 0 °C for 10 min. Within 90 min, sodium nitrite (21.0 g, 0.3 mol) was added in three times. Then, the reaction system was stirred at room temperature for 5 h. The solvent is removed under pressure and the product was dried to give a yellow solid **1** and used without further purification (Yield 93%).

**General procedure for the synthesis of compound 2.** Compound **1** was dissolved (10.0 g, 70.0 mmol) in 60 mL water and 30 mL saturated sodium bicarbonate solution was added. Within 1.5 h, sodium dithionite (34.0 g, 0.2 mol) was added to the yellow solution in batches below 20 °C. After stirring for another 90 min, the reaction was extracted with $CH_2Cl_2$ (4 × 60 mL). The organic phase was dried with sodium sulfate and the solvent was removed under reduced pressure to obtain a yellow oil **2** (Yield 52%).

**General procedure for the synthesis of compounds 3a~3q.** Compound **2** (4.72 g, 0.037 mol) and

triethyl orthoformate (6.76 g, 0.046 mol) were refluxed in MeCN for 30 min. Appropriate amine (0.041 mol) was added after the system was cooled to room temperature. The reaction mixture was stirred overnight, and the solvent was filtered to obtain solid compounds **3a~3q** (Yield 32% ~ 45%).

**General procedure for the synthesis of compounds 4a~4l and 5a~5q.** POCl$_3$ (1.2 eq) was added dropwise to a mixture of the corresponding ethyl aminobenzochromene-2-carboxylate **3** and the appropriate lactam (1.2 eq) in 1,4-dioxane (10 mL), and the solution was refluxed for 10 h. Reaction progress was monitored by thin layer chromatography (TLC). The crude product was obtained under reduced pressure and purified by silica gel chromatography and eluted with CH$_2$Cl$_2$/EtOAc to give the pure compounds **4a~4l** and **5a~5q**.

**3-Phenyl-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4a):** Yield 22.2%, sandy brown solid, mp 170 - 171 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.97 (s, 1H), 7.61 (d, $J$ = 7.7 Hz, 2H), 7.52 (t, $J$ = 7.7 Hz, 2H), 7.41 (t, $J$ = 7.4 Hz, 1H), 4.12 (t, $J$ = 6.1 Hz, 2H), 2.95 (t, $J$ = 6.7 Hz, 2H), 1.97 (p, $J$ = 6.0 Hz, 2H), 1.89 (p, $J$ = 6.1 Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 159.33, 154.90, 146.08, 138.11, 133.21, 130.05, 129.56, 124.27, 117.70, 77.30, 42.66, 32.16, 21.74, 18.85. ESI-HRMS C$_{15}$H$_{15}$N$_4$O ([M+H]$^+$): calcd for 267.1240, found 267.1240.

**3-(*p*-Tolyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4b):** Yield 28.7%, yellow solid, mp 179 - 180 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.99 (s, 1H), 7.48 (d, $J$ = 8.2 Hz, 2H), 7.32 (d, $J$ = 8.0 Hz, 2H), 4.13 (t, $J$ = 6.1 Hz, 2H), 2.95 (t, $J$ = 6.7 Hz, 2H), 2.41 (s, 3H), 1.97 (p, $J$ = 6.0 Hz, 2H), 1.91 (q, $J$ = 6.4 Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.04, 156.21, 146.55, 139.16, 138.39, 131.31, 130.43, 123.93, 120.20, 42.29, 32.07, 21.84, 21.17, 18.95. ESI-HRMS C$_{16}$H$_{16}$N$_4$O ([M+H]$^+$): calcd for 281.1397, found 281.1395.

**3-(4-Methoxyphenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4c):** Yield 24.2%, yellow solid, mp 166 - 167 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (s, 1H), 7.52 (d, $J$ = 8.9 Hz, 2H), 7.05 (d, $J$ = 8.9 Hz, 2H), 4.15 (t, $J$ = 6.2 Hz, 2H), 3.87 (s, 3H), 2.97 (t, $J$ = 6.7 Hz, 2H), 1.99 (q, $J$ = 6.4 Hz, 2H), 1.92 (q, $J$ = 6.5 Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 159.64, 157.28, 157.04, 146.90, 138.77, 127.13, 125.46, 121.67, 114.94, 55.64, 42.09, 32.02, 21.91, 19.01. ESI-HRMS C$_{15}$H$_{14}$N$_4$O([M+H]$^+$): calcd for 297.1346, found 297.1346.

**3-(4-Fluorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4d):** Yield 26.3%, white solid, mp 170 - 171 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.39 (s, 1H), 7.68 (dd, $J$ = 8.8, 4.6 Hz, 2H), 7.28 (s, 1H), 7.24 (s, 1H), 4.15 (t, $J$ = 6.1 Hz, 2H), 2.99 (t, $J$ = 6.6 Hz, 2H), 2.03 – 1.97 (m, 2H), 1.94 (q, $J$ = 6.3 Hz, 2H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 163.54, 161.06, 157.75, 156.80, 146.74, 138.49, 130.25, 125.96, 125.88, 116.94, 116.71, 42.24, 32.05, 21.88, 18.98. ESI-HRMS C$_{15}$H$_{14}$FN$_4$O ([M+H]$^+$): calcd for 285.1146, found 285.1145.

**3-(4-Bromophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4e):** Yield 22.3%, light yellow

solid, mp 171 - 172 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09 (s, 1H), 7.71 – 7.65 (m, 2H), 7.60 – 7.54 (m, 2H), 4.14 (t, $J$ = 6.1 Hz, 2H), 2.97 (t, $J$ = 6.7 Hz, 2H), 2.00 (p, $J$ = 5.8 Hz, 2H), 1.96 – 1.89 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.42, 157.17, 146.71, 138.11, 133.49, 132.92, 125.20, 122.45, 122.13, 42.17, 32.04, 21.90, 19.01. ESI-HRMS C$_{15}$H$_{14}$BrN$_4$O ([M+H]$^+$): calcd for 345.0346, found 345.0345.

**3-(4-Chlorophenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (4f):** Yield 26.1%, yellow solid, mp 195 - 196 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.92 (s, 1H), 7.63 – 7.57 (m, 2H), 7.55 – 7.50 (m, 2H), 4.16 (t, $J$ = 6.2 Hz, 2H), 2.98 (t, $J$ = 6.7 Hz, 2H), 2.04 – 1.89 (m, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.48, 157.13, 146.87, 138.07, 134.09, 133.15, 129.93, 124.87, 123.10, 42.08, 32.02, 21.93, 19.04. ESI-HRMS C$_{15}$H$_{14}$ClN$_4$O ([M+H]$^+$): calcd for 301.0851, found 301.0850.

**3-(2,4-Dichlorophenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (4g):** Yield 27.3%, light yellow solid, mp 203 - 204 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (s, 1H), 7.62 (d, $J$ = 2.0 Hz, 1H), 7.46 – 7.39 (m, 2H), 4.48 – 4.40 (m, 2H), 3.03 – 2.95 (m, 2H), 1.81 (tdd, $J$ = 11.9, 9.4, 8.6, 5.0 Hz, 6H). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 162.13, 157.33, 147.42, 139.42, 136.12, 132.24, 130.73, 130.40, 129.97, 128.26, 122.08, 42.83, 37.66, 29.56, 27.68, 25.16. ESI-HRMS C$_{16}$H$_{16}$ClN$_4$O ([M+H]$^+$): calcd for 315.1007, found 315.1005.

**3-(2-Chlorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4h):** Yield 34.5%, yellow solid, mp 217 - 218 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.87 (s, 1H), 7.62 – 7.59 (m, 1H), 7.51 – 7.44 (m, 3H), 4.15 (t, $J$ = 6.2 Hz, 2H), 2.92 (t, $J$ = 6.7 Hz, 2H), 1.99 (p, $J$ = 6.8 Hz, 2H), 1.89 (p, $J$ = 6.0 Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.31, 147.68, 139.57, 139.55, 131.66, 131.43, 130.82, 130.74, 129.25, 127.88, 121.69, 42.14, 31.93, 21.90, 19.01. ESI-HRMS C$_{15}$H$_{13}$ClN$_4$O ([M+H]$^+$): calcd for 301.0851, found 301.0849.

**3-(2,4-Dichlorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4i):** Yield 24.6%, light yellow solid, mp 170 - 171 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.85 (s, 1H), 7.61 (s, 1H), 7.46 – 7.40 (m, 2H), 4.13 (t, $J$ = 6.2 Hz, 2H), 2.90 (t, $J$ = 6.7 Hz, 2H), 1.97 (q, $J$ = 6.2 Hz, 2H), 1.89 (p, $J$ = 6.5 Hz, 2H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.50, 157.24, 147.65, 139.32, 136.19, 132.27, 130.68, 130.30, 130.09, 128.28, 121.73, 42.22, 31.93, 21.89, 18.99. ESI-HRMS C$_{15}$H$_{14}$N$_4$O ([M+H]$^+$): calcd for 335.0461, found 335.0461.

**3-(3,5-Dichlorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4j):** Yield 25.9%, white solid, mp 241 - 242 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.05 (s, 1H), 7.64 (d, $J$ = 1.6 Hz, 2H), 7.42 (s, 1H), 4.14 (t, $J$ = 6.1 Hz, 2H), 3.00 (t, $J$ = 6.6 Hz, 2H), 2.02 – 1.97 (m, 2H), 1.94 (q, $J$ = 6.6 Hz, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.74, 157.14, 146.69, 137.60, 136.21, 136.11, 128.31, 122.82, 121.94, 42.24, 32.06, 21.88, 18.98. ESI-HRMS C$_{15}$H$_{12}$Cl$_2$N$_4$O ([M+H]$^+$): calcd for 335.0461, found 335.0458.

**3-(2-Bromo-4-chlorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4k):** Yield 26.6%, white solid, mp 163 - 164 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.78 (d, $J$ = 2.2 Hz, 1H), 7.76 (s, 1H), 7.47

(dd, $J$ = 8.4, 2.2 Hz, 1H), 7.37 (d, $J$ = 8.4 Hz, 1H), 4.16 – 4.11 (m, 2H), 2.90 (t, $J$ = 6.7 Hz, 2H), 1.98 (p, $J$ = 6.6, 6.1 Hz, 2H), 1.89 (p, $J$ = 6.1 Hz, 2H).^{13}C NMR (100 MHz, CDCl$_3$) $\delta$ 157.44, 157.32, 147.68, 139.21, 136.35, 133.63, 132.08, 130.11, 128.82, 122.09, 122.04, 42.17, 31.91, 21.90, 19.02. ESI-HRMS C$_{15}$H$_{13}$BrClN$_4$O ([M+H]$^+$): calcd for 378.9956, found 378.9954.

**3-(4-Bromo-2-chlorophenyl)-5,6,7,8-tetrahydropyrido[1,2-*a*]purin-10(3*H*)-one (4l):** Yield 26.1%, white solid, mp 232 - 233 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.93 (s, 1H), 7.84 (d, $J$ = 2.5 Hz, 1H), 7.79 (d, $J$ = 8.6 Hz, 1H), 7.46 (dd, $J$ = 8.6, 2.5 Hz, 1H), 4.18 – 4.13 (m, 2H), 2.99 (t, $J$ = 6.7 Hz, 2H), 2.04 – 1.90 (m, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.43, 157.37, 146.76, 137.61, 135.82, 134.67, 134.61, 125.19, 122.69, 122.10, 42.16, 32.04, 21.91, 19.01. ESI-HRMS C$_{15}$H$_{13}$BrClN$_4$O ([M+H]$^+$): calcd for 378.9956, found 378.9953.

**3-Phenyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5a):** Yield 24.8%, light yellow solid, mp 153 - 154 °C; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.95 (s, 1H), 7.64 (d, $J$ = 7.8 Hz, 2H), 7.55 (t, $J$ = 7.7 Hz, 2H), 7.44 (t, $J$ = 7.5 Hz, 1H), 4.49 – 4.41 (m, 2H), 3.10 – 3.04 (m, 2H), 1.86 – 1.78 (m, 6H). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 161.65, 157.50, 146.67, 138.66, 134.66, 129.77, 128.27, 123.74, 123.01, 42.79, 37.83, 29.61, 27.80, 25.28. ESI-HRMS C$_{16}$H$_{17}$N$_4$O ([M+H]$^+$): calcd for 281.1397, found 281.1392.

**3-(*p*-Tolyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5b):** Yield 28.7%, light yellow solid, mp 154 - 155 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.92 (s, 1H), 7.52 – 7.48 (m, 2H), 7.34 (d, $J$ = 8.1 Hz, 3H), 4.49 – 4.43 (m, 2H), 3.06 (d, $J$ = 9.6 Hz, 2H), 2.43 (s, 3H), 1.82 (d, $J$ = 9.5 Hz, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.49, 157.47, 146.69, 138.74, 138.36, 132.04, 130.80, 130.26, 124.93, 123.70, 122.86, 42.71, 37.77, 29.58, 27.78, 25.25, 21.11. ESI-HRMS C$_{15}$H$_{14}$N$_4$O ([M+H]$^+$): calcd for 295.1553, found 295.1550.

**3-(4-Methoxyphenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5c):** Yield 29.2%, yellow solid, mp 171 - 172 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.66 (s, 1H), 7.58 (d, $J$ = 8.7 Hz, 2H), 7.07 (d, $J$ = 8.2 Hz, 2H), 4.44 (dd, $J$ = 5.1, 2.0 Hz, 2H), 3.87 (s, 3H), 3.06 (dd, $J$ = 2.8, 1.8 Hz, 2H), 1.88 – 1.77 (m, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 164.07, 160.54, 154.96, 146.08, 138.60, 128.91, 126.00, 121.71, 115.32, 55.87, 45.94, 43.41, 37.99, 29.61, 27.66, 25.13. ESI-HRMS C$_{17}$H$_{19}$N$_4$O$_2$ ([M+H]$^+$): calcd for 311.1503, found 311.1501.

**3-(4-Fluorophenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5d):** Yield 25.7%, yellow solid, mp 174 - 175 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.91 (s, 1H), 7.64 – 7.58 (m, 2H), 7.25 – 7.21 (m, 2H), 4.46 (d, $J$ = 8.3 Hz, 2H), 3.09 – 3.04 (m, 2H), 1.88 – 1.77 (m, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.80, 147.62, 146.07, 145.42, 138.51, 125.70, 125.62, 116.83, 116.60, 77.30, 42.77, 37.78, 29.55, 27.73, 25.22. ESI-HRMS C$_{15}$H$_{14}$N$_4$O ([M+H]$^+$): calcd for 299.1303, found 299.1299.

**3-(4-Bromophenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5e):** Yield 25.6%, sandy brown solid, mp 190 - 191 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.91 (s, 1H), 7.68 – 7.63 (m, 2H), 7.55 –

7.51 (m, 2H), 4.47 – 4.41 (m, 2H), 3.08 – 3.02 (m, 2H), 1.85 – 1.76 (m, 6H).^{13}C NMR (100 MHz, CDCl$_3$) $\delta$ 161.88, 157.33, 146.52, 140.23, 138.11, 133.64, 132.90, 125.12, 121.98, 42.79, 37.80, 29.55, 27.72, 25.20.  ESI-HRMS cacld for C$_{16}$H$_{16}$BrN$_4$O ([M+H]$^+$): calcd for 359.0502, found 359.0498.

**3-(4-Chlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5f):** Yield 26.4%, yellow solid, mp 191 - 192 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 7.92 (s, 1H), 7.62 – 7.58 (m, 2H), 7.53 – 7.48 (m, 2H), 4.49 – 4.38 (m, 2H), 3.10 – 3.00 (m, 2H), 1.81 (ddt, $J$ = 13.2, 8.2, 4.8 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) $\delta$ 161.87, 157.34, 146.56, 138.20, 134.09, 133.12, 129.91, 124.85, 123.01, 42.78, 37.79, 29.54, 27.71, 25.20. ESI-HRMS C$_{15}$H$_{14}$N$_4$O ([M+H]$^+$): calcd for 315.1007, found 315.0990.

**3-(3-Chlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5g):** Yield 29.6%, yellow solid, mp 209 - 210 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 7.94 (s, 1H), 7.71 (t, $J$ = 2.0 Hz, 1H), 7.55 (ddd, $J$ = 8.0, 2.1, 1.2 Hz, 1H), 7.47 (t, $J$ = 8.0 Hz, 1H), 7.43 – 7.39 (m, 1H), 4.48 – 4.41 (m, 2H), 3.12 – 3.03 (m, 2H), 1.89 – 1.75 (m, 6H).^{13}C NMR (150 MHz, CDCl$_3$) $\delta$ 162.00, 157.35, 146.54, 138.17, 135.68, 135.41, 130.78, 128.34, 123.81, 123.08, 121.61, 42.84, 37.82, 29.58, 27.73, 25.23. ESI-HRMS C$_{15}$H$_{14}$N$_4$O ([M+H]$^+$): calcd for 315.1007, found 315.1005.

**3-(2-Chlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5h):** Yield 33.4%, yellow solid, mp 185 - 186 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 7.81 (s, 1H), 7.62 – 7.58 (m, 1H), 7.48 – 7.44 (m, 3H), 4.49 – 4.41 (m, 2H), 3.04 – 2.97 (m, 2H), 1.80 (qq, $J$ = 10.0, 5.6, 4.4 Hz, 6H).^{13}C NMR (150 MHz, CDCl$_3$) $\delta$ 161.99, 157.35, 146.53, 138.17, 135.68, 135.40, 130.78, 128.34, 123.80, 123.08, 121.61, 42.83, 37.81, 29.57, 27.72, 25.22. ESI-HRMS C$_{15}$H$_{14}$N$_4$O([M+H]$^+$): calcd for 315.1007, found 315.0989.

**3-(2,4-Dichlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5i):** Yield 27.3%, light yellow solid, mp 176 - 177 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (s, 1H), 7.62 (d, $J$ = 2.0 Hz, 1H), 7.46 – 7.39 (m, 2H), 4.48 – 4.40 (m, 2H), 3.03 – 2.95 (m, 2H), 1.81 (tdd, $J$ = 11.9, 9.4, 8.6, 5.0 Hz, 6H). ^{13}C NMR (150 MHz, CDCl$_3$) $\delta$ 162.13, 157.33, 147.42, 139.42, 136.12, 132.24, 130.73, 130.40, 129.97, 128.26, 122.08, 42.83, 37.66, 29.56, 27.68, 25.16. ESI-HRMS C$_{15}$H$_{14}$N$_4$O([M+H]$^+$): calcd for 349.0617, found 349.0618.

**3-(3,5-Dichlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5j):** Yield 25.3%, white solid, mp 229 - 230 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 8.05 (s, 1H), 7.65 (d, $J$ = 1.4 Hz, 2H), 7.42 (s, 1H), 4.46 – 4.41 (m, 2H), 3.11 – 3.06 (m, 2H), 1.84 (s, 4H), 1.81 – 1.76 (m, 2H).^{13}C NMR (100 MHz, CDCl$_3$) $\delta$ 162.43, 157.03, 146.38, 137.86, 136.18, 136.06, 128.26, 122.77, 121.91, 42.91, 37.79, 29.51, 27.62, 25.13. ESI-HRMS C$_{16}$H$_{14}$Cl$_2$N$_4$O ([M+H]$^+$): calcd for 349.0617, found 349.0617.

**3-(2-Bromo-4-chlorophenyl)-3,5,6,7,8,9-hexahydro-11***H***-azepino[1,2-*a*]purin-11-one (5k):** Yield 34.6%, yellow solid, mp 161 - 162 °C; ^{1}H NMR (400 MHz, CDCl$_3$) $\delta$ 7.76 (d, $J$ = 1.5 Hz, 2H), 7.47 (dd, $J$ = 8.5, 2.2 Hz, 1H), 7.38 (d, $J$ = 8.5 Hz, 1H), 4.47 – 4.37 (m, 2H), 3.01 – 2.94 (m, 2H), 1.79 (dt, $J$ = 10.6, 4.8 Hz, 6H).^{13}C NMR (100 MHz, CDCl$_3$) $\delta$ 162.19, 157.39, 147.46, 139.45, 136.40, 133.72, 132.14,

130.20, 128.92, 122.12, 122.03, 42.87, 37.70, 29.61, 27.74, 25.22. ESI-HRMS $C_{16}H_{15}BrClN_4O$ ([M+H]$^+$): calcd for 393.0112, found 393.0112.

**3-(4-Bromo-2-chlorophenyl)-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5l):** Yield 28.0%, yellow solid, mp 160 - 161 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (d, *J* = 2.4 Hz, 2H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 4.49 – 4.42 (m, 2H), 3.03 – 2.96 (m, 2H), 1.79 (qd, *J* = 10.6, 7.4, 5.5 Hz, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.17, 157.25, 146.45, 137.76, 135.79, 134.65, 134.57, 125.13, 123.09, 122.70, 122.10, 42.84, 37.79, 29.52, 27.66, 25.16. ESI-HRMS $C_{16}H_{15}BrClN_4O$ ([M+H]$^+$): calcd for 393.0112, found 393.0110.

**3-Nonyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5m):** Yield 22.3%, yellow solid, mp 99 - 100 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.62 (s, 1H), 4.40 – 4.31 (m, 2H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.04 – 2.97 (m, 2H), 1.84 – 1.74 (m, 6H), 1.73 – 1.66 (m, 2H), 1.26 (s, 4H), 1.20 (d, *J* = 14.6 Hz, 8H), 0.81 (t, *J* = 6.8 Hz, 3H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.81, 157.40, 146.96, 139.34, 121.97, 43.60, 42.52, 37.62, 31.73, 30.11, 29.49, 29.31, 29.10, 28.93, 27.73, 26.47, 25.20, 22.55, 14.02. ESI-HRMS $C_{19}H_{31}N_4O$ ([M+H]$^+$): calcd for 331.2492, found 331.2489.

**3-Dodecyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5n):** Yield 28.4%, yellow solid, mp 94 - 95 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.63 (s, 1H), 4.43 – 4.35 (m, 2H), 4.06 (t, *J* = 7.2 Hz, 2H), 3.06 – 2.99 (m, 2H), 1.79 (tq, *J* = 9.7, 5.8, 4.4 Hz, 8H), 1.25 (d, *J* = 29.0 Hz, 18H), 0.84 (t, *J* = 6.8 Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.78, 157.39, 146.97, 139.29, 122.10, 43.60, 42.52, 37.69, 31.86, 29.56, 29.49, 29.39, 29.28, 28.97, 27.79, 26.52, 25.25, 22.63, 14.07. ESI-HRMS $C_{22}H_{37}N_4O$ ([M+H]$^+$): calcd for 373.2962, found 373.2959.

**3-Tetradecyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5o):** Yield 26.1%, white solid, mp 94 - 95 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66 (s, 1H), 4.46 – 4.39 (m, 2H), 4.08 (t, *J* = 7.2 Hz, 2H), 3.09 – 3.03 (m, 2H), 1.89 – 1.74 (m, 8H), 1.31 (dd, *J* = 9.8, 3.1 Hz, 4H), 1.25 (s, 18H), 0.87 (t, *J* = 6.8 Hz, 3H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.76, 157.36, 146.95, 139.28, 122.09, 43.57, 42.49, 37.66, 31.85, 29.61, 29.58, 29.55, 29.53, 29.47, 29.38, 29.28, 27.77, 26.50, 25.23, 22.62, 14.06. ESI-HRMS $C_{24}H_{41}N_4O$ ([M+H]$^+$): calcd for 401.3275, found 401.3270.

**3-Hexadecyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5p):** Yield 32.4%, white solid, mp 87 - 88 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.65 (s, 1H), 4.44 – 4.38 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.07 – 3.02 (m, 2H), 1.86 – 1.79 (m, 6H), 1.78 – 1.73 (m, 2H), 1.29 (d, *J* = 9.6 Hz, 4H), 1.23 (s, 22H), 0.86 (t, *J* = 6.8 Hz, 3H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.79, 157.39, 146.97, 139.29, 122.09, 43.60, 42.53, 37.69, 31.88, 30.15, 29.64, 29.61, 29.57, 29.56, 29.50, 29.40, 29.32, 28.98, 27.79, 26.53, 25.25, 22.65, 14.08. ESI-HRMS $C_{26}H_{45}N_4O$ ([M+H]$^+$): calcd for 429.3588, found 429.3584.

**3-Octadecyl-3,5,6,7,8,9-hexahydro-11*H*-azepino[1,2-*a*]purin-11-one (5q):** Yield 23.7%, yellow solid, mp 94 - 95 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66 (s, 1H), 4.42 (d, *J* = 4.8 Hz, 2H), 4.08 (t, *J* = 7.2 Hz,

2H), 3.08 – 3.04 (m, 2H), 1.85 – 1.80 (m, 6H), 1.30 (d, $J$ = 9.1 Hz, 4H), 1.24 (s, 28H), 0.87 (t, $J$ = 6.8 Hz, 3H).$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.81, 157.41, 146.98, 139.32, 122.07, 43.62, 42.55, 37.69, 31.89, 30.16, 29.66, 29.62, 29.58, 29.56, 29.51, 29.41, 29.32, 28.98, 27.79, 26.54, 25.25, 22.66, 14.09. ESI-HRMS C$_{28}$H$_{49}$N$_4$O ([M+H]$^+$): calcd for 457.3901, found 457.3895.

**Materials.** Doxorubicin was purchased from BBI Inc. (Shanghai, China). The human cancer MCF-7, HeLa, A549, and L-02 cell lines were obtained from Chinese Type Culture Collection, CAS (Shanghai, China).

**Cytotoxicity assay.** The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (GIBICO, USA) containing 10% heat-inactivated fetal bovine serum (GIBICO, USA) and antibiotic mix (1 × 100 μM penicillin A and 100 μM of streptomycin) (GIBCO, USA) in a humidified atmosphere with 95% air 5% CO$_2$ at 37 °C and have been fed every 3–4 days. Cells were seeded in 96 well plates at a certain density (HeLa cells 2 × 10$^3$ cells/well, HT-29 cells 3.5 × 10$^3$ cells/well, MCF-7 cells 4 × 10$^3$ cells/well, and L-02 cells 2.5 × 10$^3$ cells/well), then cells were treated with different compounds (50 μM) for 24 h. MTT (5 mg/mL) reagent was added 4 h before the end of culture. The medium was removed and 150 μL DMSO was added. Absorbance was measured using a microplate reader at 490 nm. IC$_{50}$ values were calculated with the inhibition rate. If the inhibition rate of the measured compound is greater than 50%, the IC$_{50}$ value is further tested. Inhibition rate = (OD value of control group experiment group) / (OD value of control group–OD value of blank group).

**REFERENCES AND NOTES**

1.  H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, and F. Bray, *CA: Cancer J. Clin.*, 2021, **71**, 209.

2.  M. S. D'Arcy, *Cell Biol. Int.*, 2019, **43**, 582.

3.  S. Senapati, A. K. Mahanta, S. Kumar, and P. Maiti, *Signal Transduct. Target. Ther.*, 2018, **3**, 7.

4.  J. D. Yang, P. Hainaut, G. J. Gores, A. Amadou, A. Plymoth, and L. R. Roberts, *Nat. Rev. Gastroenterol. Hepatol.*, 2019, **16**, 589.

5.  A. Junker, C. Renn, C. Dobelmann, V. Namasivayam, S. Jain, K. Losenkova, H. Irjala, S. Duca, R. Balasubramanian, S. Chakraborty, F. Borgel, H. Zimmermann, G. G. Yegutkin, C. E. Muller, and K.

A. Jacobson, *J. Med. Chem.*, 2019, **62**, 3677.

6.  X. Wang, Q. He, K. Wu, T. Guo, X. Du, H. Zhang, L. Fang, N. Zheng, Q. Zhang, and F. Ye, *Eur. J. Med. Chem.*, 2019, **179**, 218.

7.  V. Rep, M. Piskor, H. Simek, P. Misetic, P. Grbcic, J. Padovan, V. Gabelica Markovic, D. Jadresko, K. Pavelic, S. Kraljevic Pavelic, and S. Raic-Malic, *Molecules*, 2020, **25**, 1570.

8.  A. Nocentini, S. Bua, C. L. Lomelino, R. McKenna, M. Menicatti, G. Bartolucci, B. Tenci, L. Di Cesare Mannelli, C. Ghelardini, P. Gratteri, and C. T. Supuran, *ACS Med. Chem. Lett.*, 2017, **8**, 1314.

9.  F. Calzaferri, P. Narros-Fernandez, R. de Pascual, A. M. G. de Diego, A. Nicke, J. Egea, A. G. Garcia, and C. de Los Rios, *J. Med. Chem.*, 2021, **64**, 2272.

10. G. Faudone, S. Arifi, and D. Merk, *J. Med. Chem.*, 2021, **64**, 7156.

11. G. Dilek, I. O. Tekin, B. Coban, A. Disli, and Z. Gercek, *Med. Chem. Res.*, 2020, **30**, 84.

12. A. Y. Hassan, M. T. Sarg, A. H. Bayoumi, and F. G. A. Kalaf, *J. Heterocycl. Chem.*, 2017, **54**, 3458.

13. W. Liu, Z. Wang, F. Xu, Q. Li, H. Wang, Q. Bian, and F. Hu, *ACS Omega*, 2019, **4**, 15742.

14. S. M. Rida, F. A. Ashour, S. A. El-Hawash, M. M. El-Semary, and M. H. Badr, *Arch. Pharm.*, 2007, **340**, 185.

15. W. L. Wu, J. Hao, M. Domalski, D. A. Burnett, D. Pissarnitski, Z. Zhao, A. Stamford, G. Scapin, Y. D. Gao, A. Soriano, T. M. Kelly, Z. Yao, M. A. Powles, S. Chen, H. Mei, and J. Hwa, *ACS Med. Chem. Lett.*, 2016, **7**, 498.

16. Y. Zeng, L. Nie, K. Bozorov, Z. Ruzi, B. Song, J. Zhao, and H. A. Aisa, *J. Heterocycl. Chem.*, 2021, 1.

17. Z. Ruzi, L. Nie, K. Bozorov, J. Zhao, and H. A. Aisa, *Arch. Pharm.*, 2021, **354**, 2000470.

18. M. Felber, M. Bauwens, J. M. Mateos, S. Imstepf, F. M. Mottaghy, and R. Alberto, *Chem. Eur. J.*, 2015, **21**, 6090.

19. L. F. Nie, G. Huang, K. Bozorov, J. Zhao, C. Niu, S. S. Sagdullaev, and H. A. Aisa, *Heterocycl. Commun.*, 2018, **24**, 43.