·论 文·

含氟2,5-二酮哌嗪衍生物的设计、合成及细胞毒活性

汤 勇[1,2],廖升荣[1],李晋昇[1,2],刘永宏[1*]

([1]中国科学院南海海洋研究所,广州 510301;[2]中国科学院大学,北京 100049)

摘 要 以*N*,*N*-二乙酰基-2,5-二酮哌嗪、烯丙基溴、邻氟苯甲醛和其他芳香醛为原料,合成得到21个新型的含氟2,5-二酮哌嗪衍生物(**2a**~**2u**),其结构经^{1}H NMR、^{13}C NMR和HRMS确证。采用CCK8法初步测试了目标化合物对10株肿瘤细胞(K562,U937,MOLT-4,HL60,HeLa,DU145,MCF-7,A549,SGC-7901和H1975)的体外抑制活性。结果表明:目标化合物**2a**,**2d**,**2e**,**2f**,**2g**,**2h**,**2k**和**2t**均显示了良好的广谱的细胞毒活性,其中化合物**2t**对U937、HeLa和DU145细胞的半数抑制浓度(IC_{50})分别为0.2、0.5和0.7 μmol/L,其作为潜在的抗肿瘤活性先导化合物值得进一步深入研究。

关键词 2,5-二酮哌嗪衍生物;含氟;合成;细胞毒活性

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Design, synthesis and cytotoxic activities of fluorine-containing 2, 5-diketopiperazine derivatives

TANG Yong[1,2], LIAO Shengrong[1], LI Jinsheng[1,2], LIU Yonghong[1*]

[1] *South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301;*

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

Abstract Twenty-one novel fluorine-containing 2, 5-diketopiperazine derivatives (**2a-2u**) were synthesized by using *N*, *N*-diacetyl-2, 5-diketopiperazine, allylbromide, 2-fluorobenzaldehyde and other aromatic aldehydes. The structures were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The cytotoxicities were evaluated against ten human tumour cell lines (K562, U937, MOLT-4, HL60, HeLa, DU145, MCF-7, A549, SGC-7901, H1975) by using CCK8 assay. Results showed that compounds **2a**, **2d**, **2e**, **2f**, **2g**, **2h**, **2k**, and **2t** showed significant cytotoxicity against U937 (IC_{50} = 0.2 μmol/L), HeLa (IC_{50} = 0.5 μmol/L), and DU145(IC_{50} = 0.7 μmol/L), respectively. Compound **2t** could become a lead compound for further development for anticancer agents.

Key words 2, 5-diketopiperazine derivative; fluorine-containing; synthesis; cytotoxic activity

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2,5-二酮哌嗪化合物(2,5-diketopiperazines, DKPs,图1)常见于天然产物中[1-2],因其具有抗肿瘤[3-5]、抗菌[6]、抗污损[7]、抗病毒[8]、催产素拮抗剂[9]等生物活性而备受关注[10]。如经海洋真菌*Aspergillus sp.* 代谢产物phenylahistin结构修饰而得到的衍生物plinabulin(NPI-2358/KPU-2,图1)是一种新型的肿瘤血管破坏剂(VAD)[11],目前该药物正处于临床Ⅲ期研究阶段。Hayashi等[4]在plinabulin苯环上引入氟原子取代后得到的衍生物**33**(图1),对肿瘤细胞HT-29半数抑制浓度(IC_{50})

收稿日期 2015-12-17 * **通信作者** Tel:020-89023174 E-mail:yonghongliu@scsio.ac.cn

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能从 plinabulin 的 14 nmol/L 提高到衍生物 **33** 的 2.6 nmol/L。对该化合物进行进一步结构修饰,将 plinabulin 苯甲酰化后的产物 KPU-105 进行氟原子取代,IC_{50}能从 KPU-105 的 1.4 nmol/L 提高到衍生物 **16j** 的 0.5 nmol/L[5]。然而当 plinabulin 的咪唑基团被其他芳香基团(如苯基)取代后,这类衍生物易形成分子间氢键并产生 π-π 堆积,导致其溶解性较差[10,12-14],因而阻碍了其进一步研究。在前期的研究工作中,本课题组以 2,5-二酮哌嗪为骨架,设计将其中的一个酰胺氮原子进行烯丙基化,试图破坏其分子间氢键,并通过烯丙基的扰动,阻止分子间的 π-π 堆积,合成了一系列易溶性的 2,5-二酮哌嗪衍生物[3],研究结果发现用 2-甲氧基苯甲醛修饰的衍生物 **4m** 具有良好的广谱细胞毒活性。

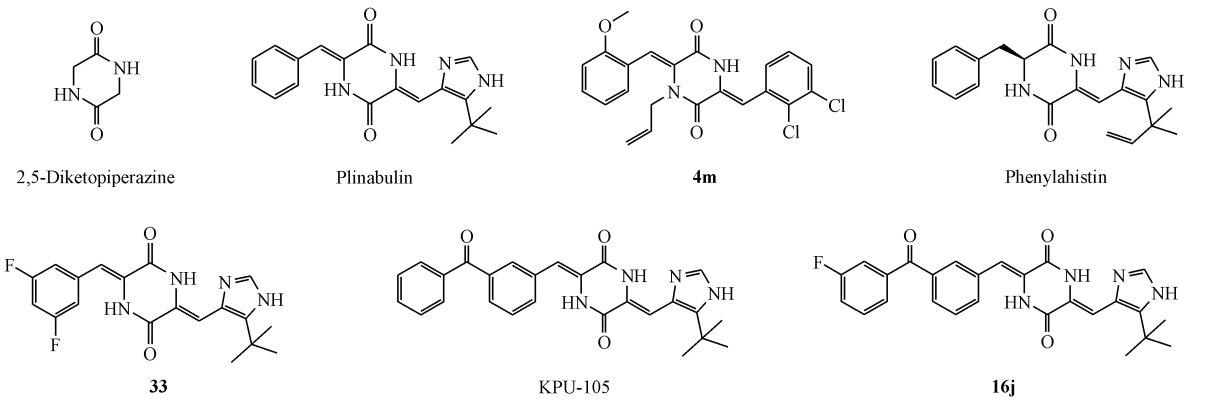
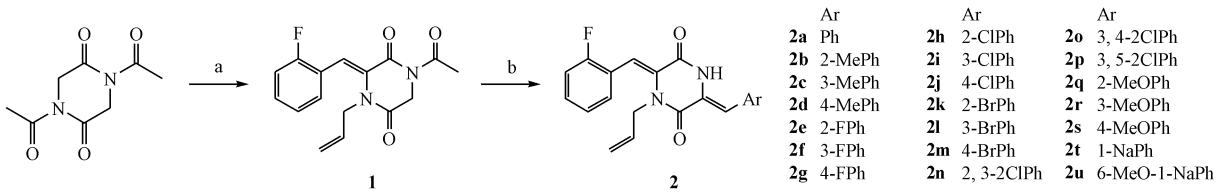


Figure 1　Structures of 2,5-diketopiperazine and its derivatives

以氟原子有利于提高药物的药效活性为启发[15-17],将衍生物 **4m** 中的邻位甲氧基用氟原子取代(路线 1),希望开发出细胞毒活性更强的 2,5-二酮哌嗪衍生物的先导化合物。本研究合成了一系列新颖的含氟 2,5-二酮哌嗪衍生物,并研究了其对 10 株肿瘤细胞(K562,U937,MOLT-4,HL60,HeLa,DU145,MCF-7,A549,SGC-7901 和 H1975)细胞毒活性。

1　合成路线

以 *N*,*N*-二乙酰基-2,5-二酮哌嗪、邻氟苯甲醛、烯丙基溴为原料,在 Cs_2CO_3 碱性条件下,通过一锅法合成(*Z*)-1-乙酰基-3-(2-氟苄叉)-4-烯丙基-2,5-二酮哌嗪中间体 **1**[18]。然后中间体 **1** 再与不同醛在 Cs_2CO_3 存在下反应,合成得到 21 个新型的含氟 2,5-二酮哌嗪化合物。具体合成路线见线路 1,化合物结构经^{1}H NMR 、^{13}C NMR 和 HRMS 确证。



Scheme 1　Synthesis of the fluorine-containing 2,5-diketopiperazine derivatives

Reagents and conditions: a) allylbromide and 2-fluorobenzaldehyde, Cs_2CO_3, DMF, r. t., 8 h; b) aromatic aldehydes, Cs_2CO_3, DMF, r. t., 3 h

2　实验部分

2.1　仪器与试剂

AV-500 型超导核磁共振波谱仪(TMS 为内标)、maXis 型高分辨飞行时间质谱仪(德国 Bruker 公司);熔点用毛细管法在 SGWX-4 显微熔点仪(上海仪电物理光学仪器有限公司)中测定(温度未校正)。

硅胶(200~300 目)和 TLC 板(青岛海洋化工厂);trichostatin A[(TSA,西格玛奥德里奇(上海)贸易有限公司];烯丙基溴和所用醛(阿拉丁试剂公司);所有试剂与溶剂均为市售分析纯;无水溶

剂用前按标准方法纯化。

2.2 化学合成

2.2.1 中间体(*Z*)-1-乙酰基-3-(2-氟苄叉)-4-烯丙基-2,5-二酮哌嗪(化合物**1**)的合成[17]

在10 mL反应瓶中依次加入*N*,*N*-二乙酰基-2,5-二酮哌嗪50 mg(0.25 mmol,1.0 equiv.),邻氟苯甲醛(0.63 mmol,2.5 equiv.),烯丙基溴(0.63 mmol,2.5 equiv.)和Cs_2CO_3(205 mg,0.63 mmol,2.5 equiv.),然后加入DMF 2 mL,在室温下搅拌反应8 h(TLC跟踪)。反应完成后加水50 mL,用稀HCl调pH到5~6。用乙酸乙酯(3×15 mL)萃取水层,合并萃取液,用无水硫酸钠干燥,旋蒸除溶剂后残余物经硅胶(200~300目)柱色谱(石油醚-乙酸乙酯,8∶1)纯化得黄色固体(52.7 mg,69.8%)。mp:115~117 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:7.42~7.38(1H,m,Ar-H),7.31~7.29(2H,m,Ar-H,=C-H),7.20(1H,t,*J*=7.5 Hz,Ar-H),7.13(1H,t,*J*=10.0 Hz,Ar-H),5.54~5.46(1H,m,=C-H),5.03(1H,d,*J*=10.0 Hz,=C-H),4.71(1H,d,*J*=15.0 Hz,=C-H),4.53(2H,s,CH_2),4.06(2H,d,*J*=10.0 Hz,CH_2),2.63(3H,s,CH_3);^{13}C NMR(125 MHz,$CDCl_3$) δ:171.50,164.60,163.96,160.39(d,J_{FC}=251.25 Hz),131.77(d,J_{FC}=8.75 Hz),131.30,130.99,130.54(d,J_{FC}=2.5 Hz),124.46(d,J_{FC}=3.75 Hz),121.25(d,J_{FC}=15.0 Hz),119.23(d,J_{FC}=1.25 Hz),119.18,116.19(d,J_{FC}=21.25 Hz),46.14,45.38,26.90;ESI-HRMS(*m/z*):325.095 9[M+Na]$^+$。

2.2.2 目标化合物**2a**~**2u**的合成方法 在10 mL反应瓶中依次加入中间体(*Z*)-1-乙酰基-3-(2-氟苄叉)-4-烯丙基-2,5-二酮哌嗪50 mg(0.17 mmol,1.0 equiv.),不同的芳香醛(0.20 mmol,1.2 equiv.)和Cs_2CO_3(85 mg,0.26 mmol,1.5 equiv.),然后加入DMF 2 mL,在室温下搅拌反应3 h(TLC跟踪)。反应完成后加水50 mL,用稀HCl调pH到5~6。水层用乙酸乙酯(3×15 mL)萃取,合并萃取液,用无水硫酸钠干燥,旋蒸除溶剂后残余物经硅胶柱色谱纯化得目标化合物**2a**~**2u**。

(3*Z*,6*Z*)-1-烯丙基-3-苄叉-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2a**) 黄色固体(25.9 mg,43.7%)。mp:59~61 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.06(1H,s,N-H),7.47~7.45(1H,m,Ar-H),7.44~7.41(3H,m,Ar-H),7.39~7.35(2H,m,Ar-H),7.30~7.27(1H,m,Ar-H),7.23(1H,s,=C-H),7.19(1H,t,*J*=7.5 Hz,Ar-H),7.14~7.08(2H,m,Ar-H,=C-H),5.57~5.50(1H,m,=C-H),5.03(1H,dd,*J*=10.0,1.0 Hz,=C-H),4.75(1H,dd,*J*=20.0,1.0 Hz,=C-H),4.28(2H,d,*J*=5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.23(d,J_{FC}=250.0 Hz),159.53,159.20,133.00,131.31,131.03,130.98(d,J_{FC}=1.25 Hz),130.90(d,J_{FC}=1.25 Hz),129.60,129.08,128.66,125.92,124.16(d,J_{FC}=3.75 Hz),122.32(d,J_{FC}=15.0 Hz),118.47,118.13,115.99(d,J_{FC}=21.25 Hz),114.91,47.51;ESI-HRMS(*m/z*):371.116 6[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2-甲基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2b**) 黄色固体(18.4 mg,29.9%)。mp:49~51 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:7.95(1H,s,N-H),7.37~7.32(2H,m,Ar-H),7.29~7.27(4H,m,Ar-H),7.23(1H,s,=C-H),7.19(1H,t,*J*=7.5 Hz,Ar-H),7.14(1H,s,=C-H),7.12(1H,t,*J*=9.0 Hz,Ar-H),5.58~5.50(1H,m,=C-H),5.03(1H,d,*J*=10.0 Hz,=C-H),4.74(1H,d,*J*=15.0 Hz,=C-H),4.30(2H,d,*J*=10.0 Hz,CH_2),2.34(3H,s,CH_3);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.44,159.03,137.84,131.73,131.34,131.23,130.98,130.92(d,J_{FC}=10.0 Hz),129.95,129.17,127.77,126.73,126.29,124.12(d,J_{FC}=3.75 Hz),122.40(d,J_{FC}=15.0 Hz),118.45,117.52,115.96(d,J_{FC}=10.0 Hz),114.83,47.45,20.16;ESI-HRMS(*m/z*):385.132 3[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3-甲基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2c**) 黄色固体(22.4 mg,36.4%)。mp:110~112 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.16(1H,s,N-H),7.38~7.32(2H,m,Ar-H),7.28(1H,t,*J*=5.0 Hz,Ar-H),7.23~7.22(3H,m,Ar-H,=C-H),7.20~7.17(2H,m,Ar-H),7.12(1H,t,*J*=9.0 Hz,Ar-H),7.07(1H,s,=C-H),5.57~5.49(1H,m,=C-H),5.02(1H,dd,*J*=15.0,5.0 Hz,=C-H),4.74

(1H, dd, J = 15.0, 1.5 Hz, =C-H), 4.28 (2H, d, J = 5.0 Hz, CH_2), 2.39 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.22 (d, J_{FC} = 248.75 Hz), 159.56, 159.24, 139.37, 132.93, 131.33, 130.98, 130.91, 130.89, 130.87, 130.09, 129.90, 129.44, 129.23, 125.75, 124.14 (d, J_{FC} = 2.5 Hz), 122.34 (d, J_{FC} = 13.75 Hz), 118.42 (d, J_{FC} = 3.75 Hz), 115.96 (d, J_{FC} = 21.25 Hz), 114.75, 47.47, 21.59; ESI-HRMS (m/z): 385.1323 [M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(4-甲基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2d**)　黄色固体(24.3 mg, 39.5%)。mp: 54 ~ 56 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.13 (1H, s, N-H), 7.39 ~ 7.32 (3H, m, Ar-H), 7.30 ~ 7.28 (2H, m, Ar-H, =C-H), 7.24 ~ 7.22 (2H, m, Ar-H), 7.20 ~ 7.17 (1H, m, Ar-H), 7.13 ~ 7.10 (1H, m, Ar-H), 7.07 (1H, s, =C-H), 5.57 ~ 5.49 (1H, m, =C-H), 5.02 (1H, dd, J = 10.0, 1.0 Hz, =C-H), 4.74 (1H, dd, J = 15.0, 1.0 Hz, =C-H), 4.28 (2H, d, J = 5.0 Hz, CH_2), 2.38 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.10 (d, J_{FC} = 250.0 Hz), 159.57, 159.26, 139.26, 131.24, 130.87, 130.79, 130.14, 130.0, 129.93, 129.40, 128.58, 127.81, 125.13, 124.05, 118.38 (d, J_{FC} = 16.25 Hz), 115.84 (d, J_{FC} = 21.25 Hz), 114.64, 47.37, 21.40; ESI-HRMS (m/z): 385.1323 [M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2-氟苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2e**)　黄色固体(20.4 mg, 32.8%)。mp: 60 ~ 62 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.05 (1H, s, N-H), 7.43 ~ 7.35 (3H, m, Ar-H), 7.29 ~ 7.28 (1H, m, Ar-H), 7.23 (1H, s, =C-H), 7.21 ~ 7.10 (4H, m, Ar-H), 7.06 (1H, s, =C-H), 5.58 ~ 5.50 (1H, m, =C-H), 5.03 (1H, d, J = 10.0 Hz, =C-H), 4.74 (1H, d, J = 15.0 Hz, =C-H), 4.28 (2H, d, J = 5.0 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.21 (d, J_{FC} = 250.0 Hz), 160.02 (d, J_{FC} = 248.75 Hz), 159.46, 158.85, 131.24, 131.05 (d, J_{FC} = 5.0 Hz), 130.99 (d, J_{FC} = 5.0 Hz), 130.90 (d, J_{FC} = 1.25 Hz), 130.18 (d, J_{FC} = 3.75 Hz), 129.83, 127.23, 125.06 (d, J_{FC} = 3.75 Hz), 124.17 (d, J_{FC} = 3.75 Hz), 122.26 (d, J_{FC} = 13.75 Hz), 120.66 (d, J_{FC} = 13.75 Hz), 118.56, 116.71 (d, J_{FC} = 22.5 Hz), 115.98 (d, J_{FC} = 21.25 Hz), 115.23, 111.40, 47.56; ESI-HRMS (m/z): 389.1072 [M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3-氟苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2f**)　黄色固体(19.6 mg, 31.5%)。mp: 65 ~ 67 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.19 (1H, s, N-H), 7.44 ~ 7.35 (2H, m, Ar-H), 7.29 ~ 7.28 (1H, m, Ar-H), 7.22 (1H, s, =C-H), 7.20 ~ 7.17 (2H, m, Ar-H), 7.14 ~ 7.10 (2H, m, Ar-H), 7.08 ~ 7.06 (1H, m, Ar-H), 7.03 (1H, s, =C-H), 5.57 ~ 5.49 (1H, m, =C-H), 5.03 (1H, d, J = 10.0 Hz, =C-H), 4.74 (1H, d, J = 15.0 Hz, =C-H), 4.28 (2H, d, J = 5.0 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.16 (d, J_{FC} = 246.25 Hz), 160.09 (d, J_{FC} = 250.0 Hz), 159.06, 158.82, 134.95 (d, J_{FC} = 7.5 Hz), 131.10, 131.07, 131.03, 130.95, 130.76, 129.70, 126.58, 124.18 (d, J_{FC} = 22.5 Hz), 118.47, 116.61, 115.98, 115.81, 115.67, 115.49, 115.20, 47.43; ESI-HRMS (m/z): 389.1072 [M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(4-氟苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2g**)　黄色固体(22.8 mg, 36.6%)。mp: 69 ~ 71 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (1H, s, N-H), 7.44 ~ 7.41 (2H, m, Ar-H), 7.38 ~ 7.36 (1H, m, Ar-H), 7.29 ~ 7.28 (1H, m, Ar-H), 7.22 (1H, s, =C-H), 7.20 ~ 7.19 (1H, m, Ar-H), 7.17 ~ 7.10 (3H, m, Ar-H), 7.05 (1H, s, =C-H), 5.55 ~ 5.50 (1H, m, =C-H), 5.03 (1H, d, J = 10.0 Hz, =C-H), 4.74 (1H, d, J = 15.0 Hz, =C-H), 4.26 (2H, d, J = 5.0 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.77 (d, J_{FC} = 250.0 Hz), 160.21 (d, J_{FC} = 250.0 Hz), 159.83, 159.17, 131.25, 131.02 (d, J_{FC} = 3.75 Hz), 130.87 (d, J_{FC} = 2.5 Hz), 130.75, 130.68, 129.93, 129.0 (d, J_{FC} = 3.75 Hz), 125.79, 124.20 (d, J_{FC} = 3.75 Hz), 122.20 (d, J_{FC} = 11.25 Hz), 118.53, 117.28, 116.79, 116.61, 116.0 (d, J_{FC} = 21.25 Hz), 115.13, 47.51; ESI-HRMS (m/z): 389.1072 [M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2-氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2h**)　黄色固体(21.5 mg, 33.1%)。mp: 110 ~ 112 ℃; ^{1}H NMR (500 MHz; $CDCl_3$) δ: 7.99 (1H, s, N-H), 7.50 ~ 7.49

(1H,m,Ar-H),7.45 ~7.43(1H,m,Ar-H),7.39 ~7.32(3H,m,Ar-H),7.30 ~7.28(1H,m,Ar-H),7.22(1H,s,=C-H),7.20 ~7.19(1H,m,Ar-H),7.17(1H,s,=C-H),7.14 ~7.10(1H,m,Ar-H),5.58 ~5.50(1H,m,=C-H),5.03(1H,d,J=10.0 Hz,=C-H),4.74(1H,d,J=20.0 Hz,=C-H),4.30(2H,d,J=5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.50,158.67,134.62,131.43,131.22,131.08,131.02,130.90(d,J_{FC}=1.25 Hz),130.72,130.25,129.72,129.34,127.55,127.20,124.15(d,J_{FC}=3.75 Hz),122.27(d,J_{FC}=15.0 Hz),118.61,115.98(d,J_{FC}=21.25 Hz),115.14(d,J_{FC}=23.75 Hz),47.50;ESI-HRMS(m/z):405.077 7[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3-氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2i**) 黄色固体(19.6 mg,30.2%)。mp:85 ~87 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.32(1H,s,N-H),7.41 ~7.37(3H,m,Ar-H),7.34 ~7.28(3H,m,Ar-H),7.23(1H,s,=C-H),7.19(1H,t,J=10.0 Hz,Ar-H),7.12(1H,t,J=9.0 Hz,Ar-H),7.02(1H,s,=C-H),5.57 ~5.49(1H,m,=C-H),5.03(1H,d,J=10.0 Hz,=C-H),4.74(1H,d,J=15.0 Hz,=C-H),4.28(2H,d,J=5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.94,158.91,135.50,134.74,131.17,131.10,130.88(d,J_{FC}=1.25 Hz),130.74,129.82,129.07,128.73,126.83,126.77,124.22(d,J_{FC}=3.75 Hz),122.16(d,J_{FC}=15.0 Hz),118.61,116.73,116.02(d,J_{FC}=21.25 Hz),115.43,47.55;ESI-HRMS(m/z):405.077 7[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(4-氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2j**) 黄色固体(20.5 mg,31.6%)。mp:135 ~137 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.27(1H,s,N-H),7.43 ~7.35(5H,m,Ar-H),7.30 ~7.28(1H,m,Ar-H),7.22(1H,s,=C-H),7.20 ~7.17(1H,m,Ar-H),7.14 ~7.10(1H,m,Ar-H),7.03(1H,s,=C-H),5.57 ~5.49(1H,m,=C-H),5.03(1H,d,J=10.0 Hz,=C-H),4.74(1H,d,J=15.0 Hz,=C-H),4.27(2H,d,J=10.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.88,159.04,134.92,131.39,131.21,131.09(d,J_{FC}=7.5 Hz),130.88(d,J_{FC}=1.25 Hz),130.10,129.74,128.94,126.24,124.19(d,J_{FC}=3.75 Hz),122.18(d,J_{FC}=13.75 Hz),118.57,117.00(d,J_{FC}=2.5 Hz),116.00(d,J_{FC}=21.25 Hz),115.22,47.51;ESI-HRMS(m/z):405.077 7[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2-溴苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2k**) 黄色固体(20.1 mg,27.7%)。mp:83 ~85 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:7.93(1H,s,N-H),7.69(1H,d,J=10.0 Hz,Ar-H),7.42 ~7.35(3H,m,Ar-H),7.30 ~7.28(1H,m,Ar-H),7.24 ~7.18(3H,m,Ar-H,=C-H),7.14 ~7.10(2H,m,Ar-H,=C-H),5.57 ~5.50(1H,m,=C-H),5.03(1H,d,J=10.0 Hz,=C-H),4.74(1H,d,J=20.0 Hz,=C-H),4.30(2H,d,J=5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.45,158.64,133.95,133.29,131.22,131.05(d,J_{FC}=7.5 Hz),130.92,130.42,129.71,129.43,128.18,127.00,124.74,124.15(d,J_{FC}=2.5 Hz),122.29(d,J_{FC}=15.0 Hz),118.62,117.21,115.98(d,J_{FC}=21.25 Hz),115.22,47.49;ESI-HRMS(m/z):449.027 1[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3-溴苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物**2l**) 黄色固体(21.4 mg,29.6%)。mp:85 ~87 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.21(1H,s,N-H),7.57(1H,s,Ar-H),7.48(1H,d,J=10.0 Hz,Ar-H),7.38 ~7.35(2H,m,Ar-H),7.33 ~7.28(2H,m,Ar-H),7.21(1H,s,=C-H),7.19 ~7.18(1H,m,Ar-H),7.14 ~7.11(1H,m,Ar-H),7.01(1H,s,=C-H),5.55 ~5.49(1H,m,=C-H),5.03(1H,d,J=10.0 Hz,=C-H),4.74(1H,d,J=15.0 Hz,=C-H),4.28(2H,d,J=5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC}=250.0 Hz),159.70,158.86,135.10,131.97,131.55,131.19,131.10(d,J_{FC}=8.75 Hz),130.99,130.88(d,J_{FC}=2.5 Hz),129.81,127.20,126.87,124.21(d,J_{FC}=2.5 Hz),123.65,122.18(d,J_{FC}=15.0 Hz),118.59,116.37,116.02(d,J_{FC}=21.25 Hz),115.36,47.54;ESI-HRMS(m/z):449.027 1[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(4-溴苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2m**)　黄色固体(27.5 mg, 38.0%)。mp: 150 ~ 152 ℃;^{1}H NMR (500 MHz;$CDCl_3$) δ:8.12(1H,s,N-H),7.58(2H,d,*J* = 10.0 Hz,Ar-H),7.38 ~ 7.35(1H,m,Ar-H),7.31(2H,d,*J* = 10.0 Hz,Ar-H),7.28 ~ 7.27(1H,m,Ar-H),7.21(1H,s, = C-H),7.20 ~ 7.17(1H,m,Ar-H),7.12(1H,t,*J* = 10.0 Hz,Ar-H),7.0(1H,s, = C-H),5.55 ~ 5.50(1H,m, = C-H),5.03(1H,dd,*J* = 10.0,1.0 Hz, = C-H),4.74(1H,dd,*J* = 15.0,5.0 Hz, = C-H),4.27(2H,d,*J* = 10.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC} = 250.0 Hz),159.63,158.99,132.75,131.88,131.21,131.08(d,J_{FC} = 8.75 Hz),130.89(d,J_{FC} = 2.5 Hz),130.26,129.89,126.32,124.20(d,J_{FC} = 3.75 Hz),123.13,122.20(d,J_{FC} = 11.25 Hz),118.57,116.77,116.0(d,J_{FC} = 21.25 Hz),115.22,47.52;ESI-HRMS(*m/z*):449.027 1[M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2,3-二氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2n**)　黄色固体(29.1 mg, 41.2%)。mp: 101 ~ 103 ℃;^{1}H NMR (500 MHz,$CDCl_3$) δ:8.18(1H,s,N-H),7.48(2H,d,*J* = 10.0 Hz,Ar-H),7.39 ~ 7.34(2H,m,Ar-H),7.30 ~ 7.27(2H,m,Ar-H),7.21 ~ 7.18(2H,m,Ar-H, = C-H),7.15(1H,s, = C-H),7.13 ~ 7.11(1H,m,Ar-H),5.58 ~ 5.50(1H,m, = C-H),5.04(1H,d,*J* = 10.0 Hz, = C-H),4.74(1H,d,*J* = 15.0 Hz, = C-H),4.30(2H,d,*J* = 5.0 Hz,CH_2);^{13}C NMR (125 MHz,$CDCl_3$) δ:160.21(d,J_{FC} = 248.75 Hz),159.62,158.47,134.73,133.63,132.90,131.18,131.14,131.12,130.91(d,J_{FC} = 2.5 Hz),130.82,129.56,128.00,127.72,127.55,124.20(d,J_{FC} = 3.75 Hz),122.20(d,J_{FC} = 13.75 Hz),118.71,116.02(d,J_{FC} = 21.25 Hz),115.53,114.72,47.55;ESI-HRMS(*m/z*):439.038 7[M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3,4-二氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2o**)　黄色固体(31.7 mg, 44.8%)。mp: 122 ~ 124 ℃;^{1}H NMR (500 MHz,$CDCl_3$) δ:8.12(1H,s,N-H),7.86(1H,d,*J* = 5.0 Hz,Ar-H),7.56 ~ 7.55(1H,m,Ar-H),7.51(1H,d,*J* = 10.0 Hz,Ar-H),7.40 ~ 7.36(1H,m,Ar-H),7.30 ~ 7.27(1H,m,Ar-H),7.23(1H,s, = C-H),7.20(1H,t,*J* = 5.0 Hz,Ar-H),7.13(1H,t,*J* = 10.0 Hz,Ar-H),7.00(1H,s, = C-H),5.57 ~ 5.49(1H,m, = C-H),5.04(1H,d,*J* = 10.0 Hz, = C-H),4.75(1H,d,*J* = 20.0 Hz, = C-H),4.28(2H,d,*J* = 5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:168.78,160.23(d,J_{FC} = 250.0 Hz),158.80,138.44,133.73,133.14(d,J_{FC} = 16.25 Hz),132.86,132.17,131.35,131.10,130.89,130.79,130.65,129.54(d,J_{FC} = 23.75 Hz),129.28,128.17,126.97,124.25(d,J_{FC} = 3.75 Hz),118.73,116.13,115.88(d,J_{FC} = 22.5 Hz),47.55;ESI-HRMS(*m/z*):439.038 7[M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3,5-二氯苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2p**)　黄色固体(32.2 mg, 45.5%)。mp: 133 ~ 135 ℃;^{1}H NMR (500 MHz,$CDCl_3$) δ:7.94(2H,s,N-H,Ar-H),7.59(1H,s,Ar-H),7.41 ~ 7.36(2H,m,Ar-H),7.32(1H,s, = C-H),7.30 ~ 7.27(1H,m,Ar-H),7.22 ~ 7.19(1H,m,Ar-H),7.15 ~ 7.12(1H,m,Ar-H),6.99(1H,s, = C-H),5.57 ~ 5.49(1H,m, = C-H),5.05(1H,d,*J* = 10.0 Hz, = C-H),4.75(1H,d,*J* = 15.0 Hz, = C-H),4.28(2H,d,*J* = 5.0 Hz,CH_2);^{13}C NMR (125 MHz,$CDCl_3$) δ:160.72,160.10(d,J_{FC} = 250.0 Hz),158.50,135.95,135.40,133.49,131.18(d,J_{FC} = 8.75 Hz),130.91,130.74,128.78,128.56,127.07,124.15(d,J_{FC} = 2.5 Hz),118.67,116.04,115.93,115.87,115.83,47.46;ESI-HRMS(*m/z*):439.038 7[M + Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(2-甲氧基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2q**)　黄色固体(24.4 mg,37.9%)。mp:57 ~ 59 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.62(1H,s,N-H),7.38 ~ 7.32(3H,m,Ar-H),7.28 ~ 7.27(1H,m,Ar-H),7.21(1H,s, = C-H),7.18 ~ 7.15(1H,m,Ar-H),7.12 ~ 7.06(2H,m,Ar-H, = C-H),7.05 ~ 6.98(2H,m,Ar-H),5.58 ~ 5.50(1H,m, = C-H),5.02(1H,d,*J* = 10.0 Hz, = C-H),4.74(1H,d,*J* = 15.0 Hz, = C-H),4.28(2H,d,*J* = 5.0 Hz,CH_2),3.96(3H,s,CH_3);^{13}C NMR (125 MHz,$CDCl_3$) δ:160.09(d,J_{FC} = 250.0 Hz),159.39,159.07,156.30,131.37,131.21,131.00,130.79(d,J_{FC} = 2.5 Hz),130.71,130.65,130.62,130.26,125.83,

123.97(d,J_{FC} =2.5 Hz),121.57,118.20,115.80(d,J_{FC} = 21.25 Hz),115.29,114.26,112.02,56.06,47.37;ESI-HRMS(*m/z*):401.127 2[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(3-甲氧基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2r**) 黄色油状物(21.7 mg,33.7%)。^{1}H NMR(500 MHz,$CDCl_3$) δ:8.12(1H,s,N-H),7.38 ~ 7.35(2H,m,Ar-H),7.29 ~ 7.28(1H,m,Ar-H),7.22(1H,s,=C-H),7.20 ~ 7.17(1H,m,Ar-H),7.12(1H,t,*J* = 9.0 Hz,Ar-H),7.06(1H,s,=C-H),7.01(1H,d,*J* = 10.0 Hz,Ar-H),6.91 ~ 6.89(2H,m,Ar-H),5.57 ~ 5.49(1H,m,=C-H),5.02(1H,dd,*J* = 15.0,5.0 Hz,=C-H),4.74(1H,dd,*J* = 15.0,1.0 Hz,=C-H),4.28(2H,d,*J* = 5.0 Hz,CH_2),3.83(3H,s,CH_3);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.21(d,J_{FC} = 250.0 Hz),160.41,159.44,159.13,134.28,131.30,131.02,130.95,130.89(d,J_{FC} = 2.5 Hz),130.68,130.02,126.14,124.16(d,J_{FC} = 2.5 Hz),122.32(d,J_{FC} = 15.0 Hz),120.63,118.47,117.98,115.98(d,J_{FC} = 21.25 Hz),114.90,114.61,114.32,55.48,47.50;ESI-HRMS(*m/z*):401.127 2[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(4-甲氧基苄叉)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2s**) 黄色油状物(18.7 mg,29.1%)。^{1}H NMR(500 MHz,$CDCl_3$) δ:8.04(1H,s,N-H),7.40 ~ 7.34(3H,m,Ar-H),7.30 ~ 7.28(1H,m,Ar-H),7.22(1H,s,=C-H),7.19 ~ 7.16(1H,m,Ar-H),7.11(1H,t,*J* = 10.0 Hz,Ar-H),7.05(1H,s,=C-H),6.97(2H,d,*J* = 10.0 Hz,Ar-H),5.57 ~ 5.49(1H,m,=C-H),5.02(1H,d,*J* = 10.0 Hz,=C-H),4.74(1H,d,*J* = 20.0 Hz,=C-H),4.26(2H,d,*J* = 5.0 Hz,CH_2),3.84(3H,s,CH_3);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.23(d,J_{FC} = 248.75 Hz),160.19,159.60,159.48,131.41,130.96,130.89,130.29,130.22,125.36,124.50,124.14(d,J_{FC} = 3.75 Hz),122.37(d,J_{FC} = 15.0 Hz),118.38,118.31,115.96(d,J_{FC} = 21.25 Hz),115.05,114.56;ESI-HRMS(*m/z*):401.127 2[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-(萘-1-基亚甲基)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2t**) 黄色固体(23.2 mg,34.3%)。mp:73 ~ 75 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.05(1H,s,N-H),7.99(1H,t,*J* = 5.0 Hz,Ar-H),7.90 ~ 7.87(2H,m,Ar-H),7.59(1H,s,Ar-H),7.57 ~ 7.52(4H,m,Ar-H,=C-H),7.40 ~ 7.35(1H,m,Ar-H),7.30(1H,t,*J* = 5.0 Hz,Ar-H),7.21 ~ 7.18(2H,m,Ar-H,=C-H),7.13(1H,t,*J* = 9.5 Hz,Ar-H),5.61 ~ 5.53(1H,m,=C-H),5.05(1H,dd,*J* = 10.0,1.0 Hz,=C-H),4.78(1H,dd,*J* = 15.0,0.5 Hz,=C-H),4.34(2H,d,*J* = 5.0 Hz,CH_2);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.19(d,J_{FC} = 248.75 Hz),159.34,158.93,134.03,131.61,131.32,131.00,130.93,129.86(d,J_{FC} = 8.75 Hz),129.67,128.88,127.40,127.11,126.85,126.25,125.65,124.65,124.14(d,J_{FC} = 2.5 Hz),122.38(d,J_{FC} = 13.75 Hz),118.50,116.39,115.96(d,J_{FC} = 21.25 Hz),114.94,47.52;ESI-HRMS(*m/z*):421.132 3[M+Na]$^+$。

(3*Z*,6*Z*)-1-烯丙基-3-((6-甲氧基萘-1-基)亚甲基)-6-(2-氟苄叉)-2,5-二酮哌嗪(化合物 **2u**) 黄色固体(23.8 mg,32.7%)。mp:150 ~ 152 ℃;^{1}H NMR(500 MHz,$CDCl_3$) δ:8.48(1H,s,N-H),7.86(1H,s,Ar-H),7.79 ~ 7.73(2H,m,Ar-H),7.48(1H,d,*J* = 5.0 Hz,Ar-H),7.38 ~ 7.34(1H,m,Ar-H),7.28 ~ 7.25(1H,m,Ar-H),7.21(1H,s,=C-H),7.20 ~ 7.18(1H,m,Ar-H),7.16 ~ 7.15(1H,m,Ar-H),7.14(1H,s,=C-H),7.11 ~ 7.09(2H,m,Ar-H),5.57 ~ 5.50(1H,m,=C-H),5.02(1H,d,*J* = 10.0 Hz,=C-H),4.74(1H,d,*J* = 15.0 Hz,=C-H),4.28(2H,d,*J* = 5.0 Hz,CH_2),3.91(1H,s,CH_3);^{13}C NMR(125 MHz,$CDCl_3$) δ:160.19(d,J_{FC} = 250.0 Hz),159.75,159.41,158.79,134.61,131.38,131.02,130.93,130.88(d,J_{FC} = 2.5 Hz),130.17,129.90,128.93,128.16,128.08,126.62,125.34,124.12(d,J_{FC} = 2.5 Hz),122.35(d,J_{FC} = 15.0 Hz),119.94,118.71,118.38,115.93(d,J_{FC} = 20.0 Hz),114.65,105.90,55.47,47.46;ESI-HRMS(*m/z*):451.142 8[M+Na]$^+$。

3 细胞毒活性评价

采用CCK8法测定了化合物对10株肿瘤细胞(K562,U937,MOLT-4,HL60,HeLa,DU145,MCF-7,A549,SGC-7901和H1975)的体外抑制活

性[3]。以 TSA 为阳性对照,实验结果见表 1 和表 2。

首先,对化合物 **2a** ~ **2u** 和中间体 **1** 进行了 6 株肿瘤细胞(HeLa,DU145,MCF-7,A549,SGC-7901 和 H1975)体外活性检测(表 1),发现中间体 **1** 对 6 株肿瘤细胞均无抑制活性,而化合物 **2a** ~ **2u** 表现出不同程度的抑制活性,这表明在 2,5-二酮哌嗪环的 3 位引入芳香取代基为活性的必需条件。化合物 **2a** 的 3 位取代基为苄叉,它显示了良好的抑制活性,IC_{50} 范围为 2 ~ 13 μmol/L。化合物 **2b** ~ **2d** 分别为邻、间、对甲基单取代苄叉情况,邻位取代的化合物 **2b** 活性较化合物 **2a** 减弱,而间位取代的化合物 **2c** 均无活性,对位取代的化合物 **2d** 能较好的保持广谱的细胞毒活性,但活性仍比化合物 **2a** 稍弱,这表明在苄叉上引入单取代的甲基均不利于活性的增强。化合物 **2e** ~ **2m** 分别为邻、间、对卤素(F、Cl、Br)单取代苄叉的情况,单氟取代的化合物 **2e** ~ **2g**、邻氯取代的化合物 **2h**、邻溴取代的化合物 **2k** 和间溴取代的化合物 **2l** 显示了良好的、广谱的细胞毒活性,其中邻氟取代的化合物 **2e** 比化合物 **2a** 抑制活性更强,IC_{50} 为 1 ~ 11 μmol/L。这表明邻氟取代有利于增强化合物的活性。与化合物 **2a** 的强活性相比,二氯取代苄叉的化合物 **2n** ~ **2p** 均未显示良好的抑制活性,说明在此类衍生物中,基团的性质、取代基的位置及大小等均对活性有重要影响。化合物 **2q** ~ **2s** 为单甲氧基取代的情况,只有化合物 **2q** 显示了良好的广谱抑制活性,但是比化合物 **2a** 弱。然而当取代基为萘(化合物 **2t**)时,化合物 **2t** 表现出最强的、广谱的抑制活性,特别是对 HeLa 和 DU145 细胞的 IC_{50} 分别为 0.5 和 0.7 μmol/L。而 6-甲氧基萘取代的化合物 **2u** 却无抑制活性,这说明了取代基的性质、大小等可能对化合物细胞毒活性有较大的影响。

Table 1　IC_{50} values(μmol/L) of fluorine-containing 2,5-diketopiperazine derivatives against cancer cell lines ($\bar{x} \pm s, n = 3$)

Compd.	Cell lines					
	HeLa	DU145	MCF-7	A549	SGC-7901	H1975
1	NA[a]	NA	NA	NA	NA	NA
2a	2.5 ± 0.3	3.4 ± 0.6	9.3 ± 1.5	8.4 ± 0.7	7.2 ± 1.3	10.8 ± 1.7
2b	NA	1.5 ± 1.3	16.1 ± 1.1	NA	35.3 ± 4.5	NA
2c	NA	NA	NA	NA	NA	NA
2d	3.1 ± 0.2	3.5 ± 0.4	16.1 ± 1.5	12.0 ± 3.4	13.3 ± 5.4	16.6 ± 2.6
2e	1.4 ± 0.4	1.7 ± 0.3	6.6 ± 0.5	3.5 ± 0.1	10.5 ± 0.8	7.5 ± 0.5
2f	3.1 ± 0.2	4.2 ± 0.2	13.5 ± 3.5	9.9 ± 0.3	16.4 ± 0.8	12.9 ± 3.0
2g	2.7 ± 0.2	2.5 ± 0.6	9.6 ± 0.3	8.4 ± 1.3	15.0 ± 2.2	15.3 + 1.1
2h	2.0 ± 0.9	2.4 ± 1.0	2.9 ± 0.2	7.2 ± 0.3	11.1 ± 1.0	8.5 ± 1.1
2i	8.2 ± 3.9	NA	18.2 ± 1.1	26.3 ± 8.3	39.2 ± 13.8	NA
2j	NA	NA	18.7 ± 0.4	NA	11.4 ± 0.8	NA
2k	4.5 ± 0.5	21.8 ± 14.2	9.2 ± 0.5	14.8 ± 4.4	15.0 ± 3.3	17.1 ± 0.8
2l	15.2 ± 8.9	32.6 ± 19.9	16.0 ± 1.7	19.6 ± 0.5	17 ± 2.8	19.3 ± 1.1
2m	11.1 ± 1.3	17.8 ± 7.8	19.1 ± 0.8	NA	18.1 ± 1.6	NA
2n	NA	15.4 ± 3.2	17.4 ± 2.9	NA	NA	NA
2o	NA	NA	19 ± 0.9	NA	NA	NA
2p	12.2 ± 3.6	NA	13.1 ± 3.1	13.5 ± 1.1	16.5 ± 0.7	11.7 ± 2.6
2q	10.6 ± 0.2	10.4 ± 7.5	16.2 ± 1.3	16.6 ± 1.4	16.5 ± 1.0	13.6 ± 3.2
2r	NA	12 ± 0.2	17.6 ± 1.7	NA	NA	NA
2s	21.9 ± 9.4	NA	19.4 ± 1.0	NA	24.3 ± 1.5	NA
2t	0.5 ± 0.1	0.7 ± 0.1	2.1 ± 0.1	3.8 ± 0.7	2.0 ± 0.4	5.8 ± 1.1
2u	NA	NA	NA	NA	NA	NA
TSA[b]	0.043 ± 0.003	0.034 ± 0.002	0.051 ± 0.009	0.028 ± 0.006	0.036 ± 0.002	0.041 ± 0.005

[a]NA: No activity; [b]TSA: Trichostatin A, used as positive control

其次,本研究对化合物 **2a** ~ **2u** 和中间体 **1** 进行了 4 株白血病细胞系(K562,U937,MOLT-4 和 HL60)进行了活性检测(表 2)。中间体 **1** 对 4 株白血病细胞系无抑制活性,而化合物 **2a** ~ **2u** 表现出不同程度的抑制活性,这进一步表明了哌嗪环的 3 位芳香取代为活性必需的条件。化合物 **2a** ~ **2u** 呈现出来的抑制活性规律和前面对 6 株肿瘤细胞检测结果相似。同样,哌嗪环 3 位引入邻氟苄叉基团时,衍生物的细胞毒活性提高,说明氟原子的引入有利于提高活性。令人惊奇的

是,当哌嗪环3位取代基为萘环时,衍生物**2t**对U937的IC_{50}达到0.2 μmol/L,而6-甲氧基萘取代的化合物**2u**对4株白血病细胞系仍未显示出抑制活性。

Table 2 IC_{50} values(μmol/L) of fluorine-containing 2,5-diketopiperazine derivatives against four leukemic cell lines

Compd.	Cell lines				Compd.	Cell lines			
	K562	U937	MOLT-4	HL60		K562	U937	MOLT-4	HL60
1	NA	NA	NA	NA					
2a	7.5 ±1.8	3.4 ±0.1	14.6 ±0.5	9.7 ±0.6	**2l**	10.9 ±2.2	NA	NA	18.6 ±0.8
2b	25.6 ±2.8	10.1 ±0.4	NA	NA	**2m**	9.1 ±0.6	NA	NA	NA
2c	NA	NA	NA	NA	**2n**	NA	NA	NA	NA
2d	13.4 ±5.3	4.8 ±0.3	21.8 ±9.1	11.4 ±2.3	**2o**	NA	20.0 ±0.5	NA	NA
2e	3.6 ±0.2	1.7 ±0.1	6.6 ±1.4	5.0 ±0.7	**2p**	8.0 ±0.7	9.9 ±2.1	NA	NA
2f	5.3 ±0.6	3.6 ±0.3	14.2 ±3.3	7.8 ±0.6	**2q**	14.8 ±0.6	9.7 ±0.6	45.2 ±12.0	18.8 ±0.5
2g	8.6 ±3.6	3.3 ±0.7	20.0 ±1.3	11.8 ±3.6	**2r**	13.9 ±4.2	10.8 ±0.7	NA	NA
2h	4.7 ±0.5	2.2 ±0.1	9.3 ±1.1	7.1 ±1.2	**2s**	35.5 ±17.8	25.2 ±16.1	27.0 ±2.8	17.0 ±0.2
2i	NA	NA	NA	NA	**2t**	1.8 ±0.1	0.2 ±0.1	3.3 ±0.2	3.1 ±0.3
2j	34.2 ±2.5	NA	NA	NA	**2u**	NA	NA	NA	NA
2k	5.6 ±0.2	5.2 ±0.7	11.0 ±0.1	17.7 ±1.8	**TSA**	0.142 ±0.012	0.032 ±0.005	0.017 ±0.001	0.039 ±0.002

4 结 论

本研究设计合成21个新型的含氟2,5-二酮哌嗪衍生物。采用CCK8法对10株肿瘤细胞(K562,U937,MOLT-4,HL60,HeLa,DU145,MCF-7,A549,SGC-7901和H1975)进行了体外细胞毒活性测试。结果表明,在含氟中间体**1**的哌嗪环上引入甲基、甲氧基、溴、氯、二氯取代的苄叉和6-甲氧基取代的萘环时,衍生物的活性减弱,但引入萘环和间氟取代的苄叉时,有利于提高化合物的活性。然而与间氟取代的苄叉衍生物相比,在含氟中间体**1**的哌嗪环上引入萘环的衍生物**2t**更有利于增强化合物的细胞毒活性。这些结果表明取代基团的性质,大小及取代基的位置对化合物的活性有着非常重要的影响。与前期研究的化合物**4m**相比,化合物**2t**对HeLa细胞半数抑制浓度提高了3倍,对U937细胞半数抑制浓度提高了2倍。这为进一步寻找活性优良的含氟2,5-二酮哌嗪先导化合物奠定了基础。

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