3'-甲基呋喃核苷衍生物的设计合成与抗肿瘤活性

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摘要: 以具有良好抗肿瘤活性的 3'-甲基腺苷和克拉屈滨为先导物, 根据生物电子等排原理, 设计并合成了 17 个 3'-甲基呋喃核苷类新化合物, 其结构经 ^{1}H NMR 和 MS 确证。用 MTT 方法评价了其体外抗肿瘤活性。结果表明, 目标化合物对人肺癌细胞 A549、人结肠癌细胞 LOVO 和人白血病细胞 CEM 均有不同程度的细胞毒性, 具有进一步研究的价值。

关键词: 甲基呋喃核苷; 合成; 抗肿瘤活性

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Design and synthesis of 3'-methyl-furanonucleosides and their anti-tumor activities

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Abstract: Taking 3'-Me-Ado (3'-methyladenosine) and Cladribine as the leading compounds, seventeen 3'-*C*-methyl-furanonucleosides were designed and synthesized. All the structures were confirmed by ^{1}H NMR and MS. The target compounds were tested *in vitro* against human pulmonary carcinoma A549, human colon carcinoma LOVO and human leukemia CEM by MTT assay. The results showed that these compounds possessed moderate cytotoxities.

Key words: methylfuranonucleoside; synthesis; anti-tumour activity

恶性肿瘤是严重威胁人类健康的一种常见疾病, 尽管迄今尚未发现根治肿瘤的药物, 但几十年来抗癌化疗已经取得了相当大的进展, 抗肿瘤药物层出不穷。核苷类化合物就是其中一类重要的抗肿瘤化疗药物[1−6]。

文献[7]报道在糖环上引入一些取代基 (如甲基、乙基、氰基等) 对其抗肿瘤活性有较大影响, Franchetti 等[8]发现在 3'位引入甲基时得到的 3'-甲基腺苷 (3'-Me-Ado, **1**) (图 1), 对人类骨髓细胞白血病 K562、白血病 K562IU 和乳腺癌 MCF-7 等较 1'-甲基腺苷和 2'-甲基腺苷类化合物有更好的活性。另外有研究显示核苷的 5'-位脱氧有助于提高其靶向性, 显著减少不良反应[9−11]。克拉屈滨 (**2**) (图 1) 的嘌呤环 2-位的氯原子对其活性也有较大影响[12]。

以抗肿瘤药物克拉屈滨和 3'-Me-Ado 为先导化

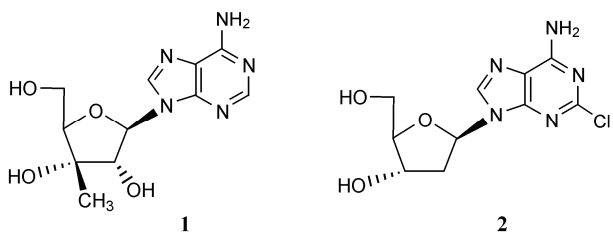

Figure 1 Chemical structures of compounds **1** and **2**

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合物, 对核苷类似物进行结构改造, 在糖环 3'-位引入甲基, 利用生物电子等排原理, 在 5'-位引入叠氮基, 设计了 17 个甲基呋喃核苷类化合物 **Ia**−**q**, 化合物结构见表 1。

以 *D*-木糖为起始原料, 经 4 步反应得到 1, 2-*O*-亚异丙基-5-*O*-叔丁基二甲硅基-*D*-3-呋喃酮糖 (**3**)。中间体 **3** 经格氏反应、羟基苯甲酰化、脱硅保护、甲磺酰化、叠氮化、去亚异丙基及乙酰化即得 1, 2-*O*-二乙酰基-3-甲基-3-*O*-苯甲酰基-5-叠氮基-*D*-呋喃木糖 (**10**)。化合物 **10** 与 6-氯嘌呤或 2, 6-二氯嘌呤在二氯化乙基铝作用下缩合得核苷类关键中间体 **11a** 和 **11b**。6-位胺化后脱保护基得目标化合物 **Ia**−**q**。合成路线图 1, 化合物结构及理化常数见表 1。

实验结果

1　化学部分

文献[13]报道若 3-羟基位于糖环平面下方时, 与 5-羟基空间距离较大, 需要使用 1, 3-二氯-1, 1, 3, 3-四异丙基二硅醚 (TIPDSCl) 在 3, 5-位形成硅醚来保护 3, 5-二羟基。若 3-羟基位于糖环平面上方时, 则与 5-羟基距离较小, 在丙酮作用下即可在 3, 5-位形成缩酮结构[14]。中间体 **4** 脱硅保护后可与丙酮成缩酮。由此推断 3-甲基在糖环下方。

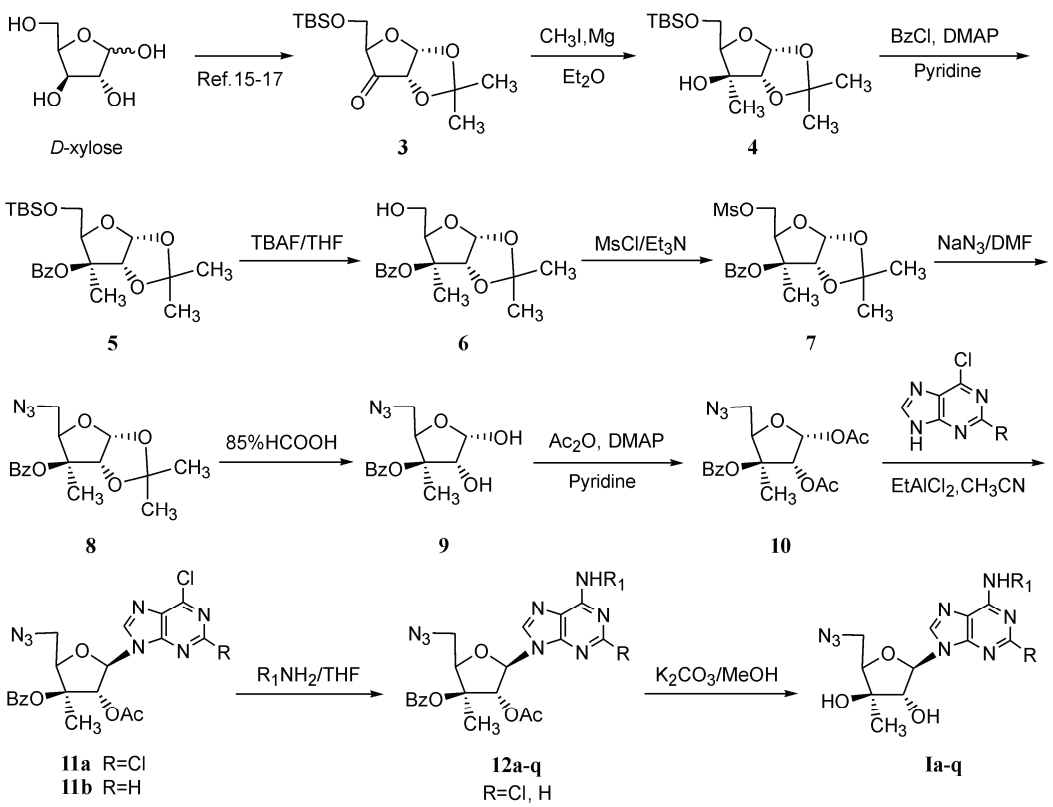
合成的 17 个目标化合物均未见文献报道, 化合物结构经 ^{1}H NMR 和 MS 确证, 图谱数据见表 2。

2　药理部分

采用 MTT 法测定 17 个目标化合物对人肺癌细胞 A549、人结肠癌细胞 LOVO 和人白血病细胞 CEM 的半数抑制浓度(IC_{50}, μg·mL^{-1}), 以克拉屈滨为阳性对照药。实验结果见表 3。

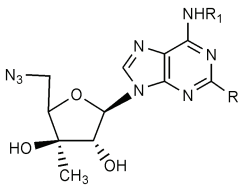
17 个目标化合物中 **Ie**、**If**、**Ih**、**Ii** 和 **Ij** 在浓度为 100 μg·mL^{-1} 时, 对 3 种肿瘤细胞均可 100%抑制; **Ie**、**If**、**Ih**、**Ii** 和 **Ij** 5 个化合物对人肺癌细胞 A549 均有一定抑制作用, IC_{50} 为 10.17～22.70 μg·mL^{-1}; **Ie**、**If**、**Ig**、**Ih**、**Ii** 和 **Ij** 6 个化合物对人结肠癌细胞 LOVO 均有一定的抑制作用, IC_{50} 为 11.09～15.81 μg·mL^{-1}; 除 **Im** 外, 其余 16 个化合物对人白血病细胞 CEM 也都有一定抑制作用, IC_{50} 为 0.372～25.38 μg·mL^{-1}, 其中以 **Ii**、**Ij** 和 **Io** 活性最好, IC_{50} 分别为 0.372、0.422 和 0.754 μg·mL^{-1}, 活性与对照药克拉屈滨差距较大。

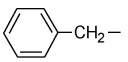
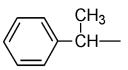
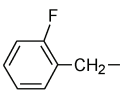
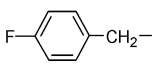
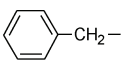
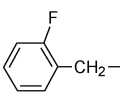
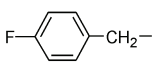
从表 3 可以看出嘌呤环 6-位取代胺基的种类与



Scheme 1　Synthetic route of target compounds

Table 1 Structures and physical properties of target compounds



Compound	R	R_1	Formular	Yield/%[a]	mp/℃	$[\alpha]_D$ [b,c]
Ia	Cl	CH_3−	$C_{12}H_{15}ClN_8O_3$	89.0	162 (dec)	+77.1°[b]
Ib	Cl	CH_3CH_2−	$C_{13}H_{17}ClN_8O_3$	86.2	168 (dec)	+63.9°[c]
Ic	Cl	$CH_3CH_2CH_2$−	$C_{14}H_{19}ClN_8O_3$	89.7	160 (dec)	+50.7°[c]
Id	Cl		$C_{14}H_{17}ClN_8O_3$	85.7	160 (dec)	+66.6°[c]
Ie	Cl		$C_{16}H_{21}ClN_8O_3$	59.6	162 (dec)	+63.4°[c]
If	Cl		$C_{17}H_{23}ClN_8O_3$	91.4	148−150	+57.1°[c]
Ig	Cl		$C_{18}H_{19}ClN_8O_3$	70.8	150 (dec)	+50.8°[b]
Ih	Cl		$C_{19}H_{21}ClN_8O_3$	65.8	158−160	+86.4°[c]
Ii	Cl		$C_{18}H_{18}ClFN_8O_3$	39.1	150 (dec)	−37.6°[b]
Ij	Cl		$C_{18}H_{18}ClFN_8O_3$	49.9	164 (dec)	+46°[c]
Ik	H	CH_3−	$C_{12}H_{16}ClN_8O_3$	68.7	160 (dec)	50.4°[b]
Il	H	CH_3CH_2−	$C_{13}H_{18}N_8O_3$	53.0	160 (dec)	50.5°[c]
Im	H		$C_{14}H_{18}N_8O_3$	38.9	178 (dec)	+55.2°[c]
In	H		$C_{16}H_{22}N_8O_3$	30.4	180 (dec)	+42.1°[c]
Io	H		$C_{18}H_{20}N_8O_3$	43.6	182 (dec)	+47.8°[c]
Ip	H		$C_{18}H_{19}FN_8O_3$	33.4	150 (dec)	−29.4°[b]
Iq	H		$C_{18}H_{19}FN_8O_3$	26.0	164 (dec)	+39.2°[c]

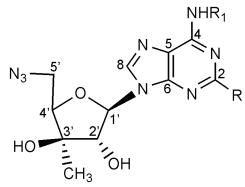
a: The yield for two steps (amination and deprotection); b: *c* 1.0 in DMSO, 20 ℃; c: *c* 1.0 in CH_3OH, 20 ℃

活性有一定关系。总体来看在本文的嘌呤核苷化合物的 6-位取代基活性顺序为苄胺或取代苄胺 > 环状脂肪胺 > 链状脂肪胺, 嘌呤环 2-位含氯原子比不含氯原子时显示出较好的活性。

结论

本研究通过以抗肿瘤药克拉屈滨和 3'-Me-Ado 为先导化合物, 在糖环 3'-位引入甲基, 在 5'-位引入叠氮基, 设计合成了 17 个甲基呋喃核苷类化合物 **Ia−q**。化合物结构经 ^{1}H NMR 和 MS 确证。评价了对人肺癌细胞 A549、人结肠癌细胞 LOVO 和人白血病细胞 CEM 的体外抗肿瘤活性: **Ie**、**If**、**Ih**、**Ii** 和 **Ij** 5 个化合物在浓度为 100 μg·mL^{-1}时, 对 3 种肿瘤细胞均可 100% 抑制; 除 **Im** 外, 其余 16 个化合物对人白血病细胞 CEM 都有一定抑制作用, IC_{50} 为 0.372～25.38 μg·mL^{-1}, 其中以 **Ii**、**Ij** 和 **Io** 活性最好, IC_{50} 分别为 0.372、0.422 和 0.754 μg·mL^{-1}。

Table 2 Spectral data of target compounds



No.	ESI-MS	^{1}H NMR δ (J in Hz)
Ia	389[M+Cl]$^-$	8.41 (1H, s, 8-H), 8.20 (1H, b, N-H), 5.81 (1H, d, J = 8.4, 1'-H), 5.54 (1H, d, J = 7.2, 2'-OH), 5.08 (1H, s, 3'-OH), 4.61 (1H, t, J = 8.4, J = 7.2, 2'-H), (4.01−4.04 (1H, m), 3.81−3.89 (1H, m), 5'-H), 3.27−3.31 (1H, m, 4'-H), 1.23 (3H, b, R_1), 1.20 (3H, s, 3'-CH_3)
Ib	391[M+Na]$^+$	8.49 (1H, s, 8-H), 8.33 (1H, b, N-H), 5.89 (1H, d, J = 8.4, 1'-H), 5.61 (1H, d, J = 7.2, 2'-OH), 5.14 (1H, s, 3'-OH), 4.68 (1H, t, J = 8.4, J = 7.2, 2'-H), (4.08−4.11 (1H, m), 3.93−3.98 (1H, m), 5'-H), 3.35−3.39 (1H, m, 4'-H), 3.53 (2H, b, R_1), 1.23 (3H, t, R_1), 1.22 (3H, s, 3'-CH_3)
Ic	405[M+Na]$^+$	8.41 (1H, s, 8-H), 8.27 (1H, b, N-H), 5.84 (1H, d, J = 8.4, 1'-H), 5.53 (1H, d, J = 6.4, 2'-OH), 5.06 (1H, s, 3'-OH), 4.60 (1H, t, J = 8.4, J = 6.4, 2'-H), (4.00−4.04 (1H, m), 3.86−3.91 (1H, m), 5'-H), 3.27−3.31 (1H, m, 4'-H), 3.38 (2H, b, R_1), 1.58−1.64 (2H, m, R_1), 0.90 (3H, t, R_1), 1.20 (3H, s, 3'-CH_3)
Id	403[M+Na]$^+$	8.42 (1H, s, 8-H), 8.38 (1H, b, N-H), 5.85 (1H, d, J = 7.6, 1'-H), 5.54 (1H, d, J = 6.8, 2'-OH), 5.06 (1H, s, 3'-OH), 4.60 (1H, t, J = 7.6, J = 6.8, 2'-H), (4.01−4.04 (1H, m), 3.86−3.91 (1H, m), 5'-H), 3.28−3.32 (1H, m, 4'-H), 3.17 (1H, b, R_1), 1.21 (3H, s, 3'-CH_3), 0.73−0.78 (2H, m, R_1), 0.60−0.64 (2H, m, R_1)
Ie	431[M+Na]$^+$	8.41 (1H, s, 8-H), 8.21 (1H, d, J = 7.6, N-H), 5.84 (1H, d, J = 8.0, 1'-H), 5.52 (1H, d, J = 6.8, 2'-OH), 5.05 (1H, s, 3'-OH), 4.60 (1H, t, J = 8.0, J = 6.8, 2'-H), (4.00−4.03 (1H, m), 3.86−3.91 (1H, m), 5'-H), 4.04 (1H, b, R_1), 3.31−3.35 (1H, m, 4'-H), 1.54−1.98 (8H, m, R_1), 1.20 (3H, s, 3'-CH_3)
If	445[M+Na]$^+$	8.41 (1H, s, 8-H), 8.07 (1H, d, J = 8.0, N-H), 5.84 (1H, d, J = 8.4, 1'-H), 5.52 (1H, d, J = 5.6, 2'-OH), 5.06 (1H, s, 3'-OH), 4.59 (1H, t, J = 8.0, J = 5.6, 2'-H), (4.00−4.04 (1H, m), 3.86−3.91 (1H, m), 5'-H), 4.00 (1H, b, R_1), 3.31−3.35 (1H, m, 4'-H), 1.12−1.99 (10H, m, R_1), 1.20 (3H, s, 3'-CH_3)
Ig	429[M−H]$^-$	8.82 (1H, b, N-H), 8.45 (1H, s, 8-H), 7.21−7.34 (5H, m, R_1), 5.86 (1H, d, J = 8.0, 1'-H), 5.54 (1H, d, J = 6.4, 2'-OH), 5.07 (1H, s, 3'-OH), 4.67 (2H, b, R_1), 4.60 (1H, t, J = 8.0, J = 6.4, 2'-H), (4.02−4.05 (1H, m), 3.85−3.91 (1H, m), 5'-H), 3.32−3.37 (1H, m, 4'-H), 1.20 (3H, s, 3'-CH_3)
Ih	443[M−H]$^-$	8.76 (1H, d, J = 7.2, N-H), 8.45 (1H, s, 8-H), 7.19−7.45 (5H, m, R_1), 5.84 (1H, d, J = 7.6, 1'-H), 5.52 (1H, d, J = 6.4, 2'-OH), 5.06 (1H, s, 3'-OH), 4.60 (1H, b, R_1), 4.60 (1H, t, J = 7.6, J = 6.4, 2'-H), (4.02−4.05 (1H, m), 3.85−3.91 (1H, m), 5'-H), 3.32−3.37 (1H, m, 4'-H), 1.55 (3H, d, J = 7.2, CH_3), 1.20 (3H, s, 3'-CH_3)
Ii*	449.133 4 (449.125 3) [M+H]$^+$	8.78 (1H, b, N-H), 8.52 (1H, s, 8-H), 7.17−7.44 (4H, m, R_1), 5.91 (1H, d, J = 8.0, 1'-H), 5.59 (1H, d, J = 7.2, 2'-OH), 5.12 (1H, s, 3'-OH), 4.77 (2H, b, R_1), 4.65 (1H, t, J = 8.0, J = 7.2, 2'-H), (4.07−4.09 (1H, m), 3.90−3.96 (1H, m), 5'-H), 3.38−3.42 (1H, m, 4'-H), 1.27 (3H, s, 3'-CH_3)
Ij*	449.131 9 (449.125 3) [M+H]$^+$	8.88 (1H, b, N-H), 8.50 (1H, s, 8-H), 7.16−7.46 (4H, m, R_1), 5.91 (1H, d, J = 7.6, 1'-H), 5.58 (1H, d, J = 6.8, 2'-OH), 5.12 (1H, s, 3'-OH), 4.69 (2H, b, R_1), 4.65 (1H, t, J = 7.6, J = 6.8, 2'-H), (4.07−4.10 (1H, m), 3.90−3.96 (1H, m), 5'-H), 3.39−3.42 (1H, m, 4'-H), 1.28 (3H, s, 3'-CH_3)
Ik	355[M+Cl]$^-$	8.37 (1H, s, 8-H), 8.25 (1H, s, 2-H), 7.63 (1H, b, N-H), 5.90 (1H, d, J = 8.0, 1'-H), 5.50 (1H, d, J = 6.8, 2'-OH), 5.01 (1H, s, 3'-OH), 4.74 (1H, t, J = 8.0, J = 6.8, 2'-H), (4.00−4.03 (1H, m), 3.92−3.95 (1H, m), 5'-H), 3.23−3.36 (1H, m, 4'-H), 2.99 (3H, b, R_1),1.22 (3H, s, 3'-CH_3)
Il	357[M+Na]$^+$	8.37 (1H, s, 8-H), 8.22 (1H, s, 2-H), 7.68 (1H, b, N-H), 5.90 (1H, d, J = 8.0, 1'-H), 5.50 (1H, d, J = 6.4, 2'-OH), 5.01 (1H, s, 3'-OH), 4.73 (1H, t, J = 8.0, J = 6.4, 2'-H), (4.00−4.03 (1H, m), 3.90−3.95 (1H, m), 5'-H), 3.56 (2H, b, R_1), 3.25−3.35 (1H, m, 4'-H), 1.21 (3H, s, 3'-CH_3), 1.17−1.20 (2H, m, R_1)
Im	381[M+Cl]$^-$	8.38 (1H, s, 8-H), 8.27 (1H, s, 2-H), 7.83 (1H, d, J = 4.0, N-H), 5.91 (1H, d, J = 8.0, 1'-H), 5.50 (1H, d, J = 6.0, 2'-OH), 5.02 (1H, s, 3'-OH), 4.73 (1H, t, J = 8.0, J = 6.0, 2'-H), (4.01−4.04 (1H, m), 3.90−3.95 (1H, m), 5'-H), 3.26−3.35 (1H, m, 4'-H), 3.09 (1H, b, R_1), 1.22 (3H, s, 3'-CH_3), , 0.71−0.76 (2H, m, R_1), 0.61−0.65 (2H, m, R_1)
In	431[M+Na]$^+$	8.37 (1H, s, 8-H), 8.22 (1H, s, 2-H), 7.57 (1H, d, J = 7.6, N-H), 5.90 (1H, d, J = 7.6, 1'-H), 5.48 (1H, d, J = 7.2, 2'-OH), 5.00 (1H, s, 3'-OH), 4.72 (1H, t, J = 7.6, J = 7.2, 2'-H), 4.70 (1H, b, R_1), (4.00−4.03 (1H, m), 3.89−3.94 (1H, m), 5'-H), 3.25−3.34 (1H, m, 4'-H), 1.54−1.99 (8H, m, R_1), 1.21 (3H, s, 3'-CH_3)
Io*	397.173 0 (397.173 7) [M+H]$^+$	8.50 (1H, s, 8-H), 8.32 (1H, s, 2-H), 8.32 (1H, b, N-H), 7.30−7.44 (5H, m, R_1), 5.98 (1H, d, J = 7.6, 1'-H), 5.59 (1H, d, J = 6.8, 2'-OH), 5.11 (1H, s, 3'-OH), 4.80 (2H, b, R_1), 4.79 (1H, d, J = 7.6, 2'-H), (4.10−4.12 (1H, m), 3.93−3.98 (1H, m), 5'-H), 3.38−3.42 (1H, m, 4'-H), 1.30 (3H, s, 3'-CH_3)
Ip	415[M+H]$^+$	8.44 (1H, s, 8-H), 8.28 (1H, b, N-H), 8.23 (1H, s, 2-H), 7.08−7.34 (4H, m, R_1), 5.91 (1H, d, J = 8.0, 1'-H), 5.53 (1H, s, 2'-OH), 5.05 (1H, s, 3'-OH), 4.78 (2H, b, R_1), 4.70 (1H, d, J = 8.0, 2'-H), (4.00−4.03 (1H, m), 3.88−3.94 (1H, m), 5'-H), 3.16−3.21 (1H, m, 4'-H), 1.20 (3H, s, 3'-CH_3)
Iq	415[M+H]$^+$	8.41 (1H, s, 8-H), 8.29 (1H, b, N-H), 8.23 (1H, s, 2-H), 7.07−7.39 (4H, m, R_1), 5.90 (1H, d, J = 8.0, 1'-H), 5.50 (1H, s, 2'-OH), 5.03 (1H, s, 3'-OH), 4.72 (2H, b, R_1), 4.72 (1H, d, J = 8.0, 2'-H), (4.00−4.03 (1H, m), 3.88−3.93 (1H, m), 5'-H), 3.10−3.27 (1H, m, 4'-H), 1.20 (3H, s, 3'-CH_3)

*HR-ESI-MS

Table 3 Inhibition against human tumor cells *in vitro*

Sample	A549		LOVO		CEM	
	IC_{50}/μg·mL^{-1}	IC% at 100 μg·mL^{-1}	IC_{50}/μg·mL^{-1}	IC% at 100 μg·mL^{-1}	IC_{50}/μg·mL^{-1}	IC% at 100 μg·mL^{-1}
Cladribine	2.12	90.12	0.201	97.98	0.0747	83.73
Ia	>100	49.84	97.06	50.46	17.91	80.23
Ib	83.00	52.20	42.76	77.80	14.98	78.49
Ic	>100	48.86	42.23	77.90	19.83	79.00
Id	>100	41.06	73.74	57.34	25.38	72.28
Ie	22.70	100	15.04	100	10.92	100
If	10.17	100	11.22	100	14.02	100
Ig	69.90	54.22	11.09	100	21.37	71.06
Ih	18.49	100	15.81	100	11.02	100
Ii	19.24	100	14.86	100	0.422	100
Ij	18.16	100	14.79	100	0.372	100
Ik	>100	32.76	96.78	50.70	14.34	79.09
Il	>100	42.13	>100	47.28	12.89	78.38
Im	>100	29.76	>100	43.27	68.67	56.65
In	>100	46.44	30.00	81.13	19.54	71.93
Io	69.81	56.78	52.09	65.40	0.754	89.57
Ip	>100	30.53	>100	29.59	11.52	77.78
Iq	>100	35.82	>100	26.61	10.94	78.70

实验部分

熔点用毛细管熔点仪测定, 温度计未校正; ^{1}H NMR 由 Varian AM-400 型核磁共振仪测定, 以 TMS 为内标; MS 用 Q-TOF 型质谱仪测定; 元素分析由 Carlo Erba1106 型自动分析仪测定; 比旋光度由 Perkin Elmer P-341 旋光仪测定。

薄层层析板为烟台芝罘硅胶开发实验厂生产的 HSGF254 型。显色一般为 Hannesion 显色剂或 254A 紫外灯下照射。无水溶剂及试剂按常规方法处理, 无水、无氧反应均在干燥溶剂中、N_2 保护下进行。

1 1,2-*O*-亚异丙基-3-甲基-5-*O*-叔丁基二甲硅基-*α*-*D*-呋喃木糖 (4)

镁屑 (12.3 g, 0.514 mol), 乙醚 (110 mL) 置于 2 L 四颈瓶中, 加入碘甲烷 (73.1 g, 0.51 mol, 以 32 mL 乙醚稀释), 加毕, 回流至镁屑消失, 降温至 20～25 ℃, 滴加 **3** 的乙醚溶液 (129.5 g, 0.43 mol, 以 50 mL 乙醚稀释), 室温搅拌过夜。饱和氯化铵溶液淬灭反应, 乙醚提取 3 次 (100 mL×3), 水洗, 无水硫酸镁干燥, 浓缩, 得 125.3 g 无色液体 (**4**)[15], 所得粗品直接用于下一步反应。$[\alpha]_D$ = +61.8° (*c* 1.0, $CHCl_3$)。

2 1,2-*O*-亚异丙基-3-甲基-3-*O*-苯甲酰基-5-*O*-叔丁基二甲硅基-*α*-*D*-呋喃木糖 (5)

125.3 g 化合物 **4** (0.412 mol) 和 630 mL 吡啶置于 1 L 三颈瓶中, 加入 DMAP (1.93 g, 0.016 mol) 后在 0 ℃下滴加苯甲酰氯 (88.5 mL, 0.473 mol), 加毕, 室温搅拌过夜。冰水浴冷却下加入少量甲醇 (约 10 mL) 破坏过量苯甲酰氯, 再加水 600 mL 稀释, 乙醚萃取, 水洗, 无水硫酸镁干燥, 过滤, 浓缩, 粗品经柱层析纯化, 石油醚-乙酸乙酯 (6∶1) 洗脱得 118.3 g 无色液体 (**5**), 收率 68.8%。MS *m/z*: 422 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$) *δ*: 0.125 (s, 3H), 0.135 (s, 3H), 0.31 (s, 3H), 0.93 (s, 9H), 1.48 (s, 3H), 1.50 (s, 3H), 3.90～3.92 (m, 2H), 4.28 (m, 1H), 4.92 (d, *J* = 3.6 Hz, 1H), 5.81 (d, *J* = 3.6Hz, 1H), 7.40～7.44 (m, 2H), 7.55 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 2H)。

3 1,2-*O*-亚异丙基-3-甲基-3-*O*-苯甲酰基-*α*-*D*-呋喃木糖 (6)

118.3 g 化合物 **5** (0.280 mol) 和 890 mL THF 置于 2 L 的茄形反应瓶中, 加入 1 mol·L^{-1} 四丁基氟化铵 (TBAF) 的 THF 溶液 (289 mL, 0.289 mol), 室温搅拌 1 h。蒸干反应液, 粗品柱层析纯化, 石油醚-乙酸乙酯 (3∶1) 洗脱得 70.6 g 白色固体 (**6**)[16], 收率 81.8%。$[\alpha]_D$ +70.9° (*c* 1.0, $CHCl_3$), mp 114～116℃。^{1}H NMR ($CDCl_3$) *δ*: 1.31 (s, 3H), 1.48 (s, 3H), 1.49 (s, 3H), 2.18 (b, 1H), 3.85～3.93 (m, 2H), 4.32～4.35 (m, 1H, 加重水消失), 4.93 (d, *J* = 3.6 Hz, 1H), 5.59 (d, *J* = 3.6 Hz, 1H), 7.41～7.44 (m, 2H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.99～8.01 (m, 2H)。

4 1,2-*O*-亚异丙基-3-甲基-3-*O*-苯甲酰基-5-*O*-甲磺酰基-*α*-*D*-呋喃木糖 (7)

6 (70.6 g, 0.23 mol), 二氯甲烷 (760 mL), 三乙胺 (63.8 mL, 0.458 mol) 置于 2 L 三颈瓶中, 冷至 0 ℃开始滴加甲磺酰氯 (40.2 mL, 0.458 mol), 加毕, 0 ℃搅拌 3 h。将反应液倾入 1 L 冰水混合物中, 二氯甲烷萃取, 饱和食盐水洗, 无水硫酸镁干燥, 过滤, 浓缩, 粗品经柱层析纯化, 石油醚-乙酸乙酯 (5∶1) 洗脱得 72.2 g 白色固体 (**7**)[17], 收率 81.8% (文献收率 95%)。$[\alpha]_D$ +70.4° (*c* 1.0, $CHCl_3$), mp 96～97 ℃。MS *m/z*: 409 $[M+Na]^+$; ^1H NMR ($CDCl_3$) *δ*: 1.32 (s, 3H), 1.49 (s, 3H), 1.51 (s, 3H), 3.10 (s, 3H), 4.39～4.49 (m, 2H), 4.54～4.57 (m, 1H), 4.96 (d, *J* = 3.6 Hz, 1H), 5.85 (d, *J* = 3.6 Hz, 1H), 7.43～7.46 (m, 2H), 7.56 (m, 1H), 8.00～8.02 (m, 2H)。

5 1,2-*O*-亚异丙基-3-甲基-3-*O*-苯甲酰基-5-叠氮基-*α*-*D*-呋喃木糖 (8)

7 (72.2 g, 0.187 mol), 干燥的 DMF (1.2 L), NaN_3 (72.9 g, 1.12 mol) 置于 2 L 三颈瓶中, 90 ℃搅拌过夜。加冰水 1 L, 乙醚萃取, 水洗, 无水硫酸镁干燥, 过滤, 浓缩, 粗品经柱层析纯化, 石油醚-乙酸乙酯 (5∶1) 洗脱得 49.4 g 白色固体 (**8**)[18], 收率 79.3%。$[\alpha]_D$ +38.7° (*c* 1.0, $CHCl_3$), mp 101～102 ℃。MS *m/z*: 356 $[M+Na]^+$; ^1H NMR ($CDCl_3$) *δ*: 1.32 (s, 3H), 1.49 (s, 3H), 1.51 (s, 3H), 3.51～3.58 (m, 2H), 4.34～4.38 (m, 1H), 4.94 (d, *J* = 3.6 Hz, 1H), 5.83 (d, *J* = 3.6 Hz, 1H), 7.42～7.46 (m, 2H), 7.55～7.59 (m, 1H), 8.00～8.02 (m, 2H)。

6 5-叠氮基-3-甲基-3-*O*-苯甲酰基-*α*-*D*-呋喃木糖 (9)

8 (49.4 g, 0.148 mol), 85%甲酸 (700 mL) 置于 1 L 茄形反应瓶中, 室温搅拌过夜。蒸干得 37.7 g 无色液体 (**9**), 粗品不经纯化直接进行下一步反应。

7 1,2-*O*-二乙酰基-3-甲基-3-*O*-苯甲酰基-5-叠氮基-*α*-*D*-呋喃木糖 (10)

37.7 g 化合物 **9** (0.129 mol), 643 mL 干燥吡啶置于 1 L 茄形反应瓶中, 加入 643 mL 乙酸酐和 0.628 g DMAP (5.14 mmol), 室温搅拌 12 h。向反应液中加入冰水混合物, 乙酸乙酯萃取, 饱和碳酸氢钠溶液洗, 水洗, 无水硫酸镁干燥, 过滤, 浓缩, 粗品经柱层析纯化, 石油醚-乙酸乙酯 (5∶1) 洗脱得 39.7 g 无色液体 (**10**)[19], 收率 81.8%。

8 2,6-二氯-9*H*-(2-*O*-乙酰基-3-甲基-3-*O*-苯甲酰基-5-叠氮基-*β*-*D*-呋喃木糖基) 嘌呤 (11a)

10 (6 g, 15.9 mmol), 2,6-二氯嘌呤 (7.37 g, 39.7 mmol), 干燥的乙腈 (60 mL) 置于 250 mL 三颈瓶中, 氮气保护下, 于 0 ℃滴加二氯化乙基铝的甲苯溶液 (17.7 mL, 0.9 mol·L^{-1}), 回流过夜。将反应液倾入冰二氯甲烷和饱和 $NaHCO_3$ 溶液的混合物中, 搅拌 5 min 后分出有机层, 水层用二氯甲烷萃取, 合并有机层, 水洗, 无水硫酸镁干燥, 过滤, 浓缩, 粗品经柱层析纯化, 石油醚-乙酸乙酯 (2∶1) 洗脱得类白色固体 **11a** (4.79 g)[19], 收率 59.6%。 $[\alpha]_D$ −26.1° (*c* 1.0, $CHCl_3$), mp 60～61 ℃。MS *m/z*: 528 $[M+Na]^+$; ^1H NMR ($CDCl_3$) *δ*: 1.81 (s, 3H), 2.20 (s, 3H), 3.80～3.85 (m, 2H), 4.86 (t, *J* = 4.4 Hz, 1H), 6.07 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 7.6 Hz, 1H), 7.44～7.48 (m, 2H), 7.59(d, *J* = 7.6 Hz, 1H), 7.96～7.98 (d, *J* = 7.6 Hz, 2H), 8.41(s, 1H)。

9 6-氯-9*H*-(2-*O*-乙酰基-3-甲基-3-*O*-苯甲酰基-5-叠氮基-*β*-*D*-呋喃木糖基) 嘌呤 (11b)

操作方法同上, 粗品经柱层析纯化得类白色固体 **11b** (4.20 g), 收率 56.1%。$[\alpha]_D$ −50.5° (*c* 1.0, $CHCl_3$), mp 56～58 ℃。MS *m/z*: 494 $[M+Na]^+$; ^1H NMR ($CDCl_3$) *δ*: 1.81 (s, 3H), 2.19 (s, 3H), 3.74～3.91 (m, 2H), 4.86 (t, *J* = 4.4 Hz, 1H), 6.26 (d, *J* = 7.6 Hz, 1H), 6.40 (d, *J* = 7.6 Hz, 1H), 7.42～7.46 (m, 2H), 7.55～7.59 (m, 1H), 8.00～8.02 (m, 2H), 8.50 (s, 1H), 8.73 (s, 1H)。

10 2-氯-N^6-甲基-9*H*-(3-甲基-5-叠氮基-*β*-*D*-呋喃木糖基) 嘌呤 (Ia)

11a (0.5 g, 0.90 mmol), THF (10 mL), 35%甲胺水溶液 (1 mL) 置于 25 mL 茄形瓶中, 40 ℃搅拌 2 h, 蒸干反应液, 得白色固体 (**12a**)。粗品不经纯化直接用于下一步反应。

将 **12a** 用 10 mL 甲醇溶解, 加入碳酸钾 (0.413 g, 2.97 mmol), 室温搅拌 2 h, 过滤, 浓缩, 粗品经柱层析纯化, 二氯甲烷-甲醇 (20∶1) 洗脱得 0.283 g 白色固体 **Ia**, 两步合并收率为 89.0%。

按照上述方法合成目标化合物 **Ia**−**q**, 波谱数据见表 2。

药理实验

采用 MTT 法测定 17 个目标化合物对人肺癌细胞 A549、人结肠癌细胞 LOVO 和人白血病细胞 CEM 的半数抑制浓度 (IC_{50}, μg·mL^{-1}), 以克拉屈滨为阳性对照药。

MTT 法: 96 孔板每孔加入 4～5×10^4 个/ mL 的细胞悬液 100 μL, 置 37 ℃, 5% CO_2 培养箱内, 24 h 后加入样品液, 每孔 10 μL, 设双复孔。37 ℃, 5% CO_2 作

用 72 h。然后每孔加入 5 mg·mL^{-1} MTT 溶液 20 μL, 作用 4 h 后加入溶解液, 每孔 100 μL, 置培养箱内, 溶解后用 MK-2 全自动酶标仪测 570 nm 吸收度值。

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