大黄酸胺基醇酯衍生物的合成及其抗骨肉瘤细胞活性

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摘要: 以大黄酸为原料, 设计、合成了 24 个大黄酸胺基醇酯衍生物。所合成的目标化合物均经 IR、HR-MS 及 ^{1}H NMR 进行结构确认。水溶性测试实验表明, 目标化合物的水溶性均有大幅提高, 水中溶解度 (10.04～15.08 mg·mL^{-1}) 是大黄酸 (0.045 6 mg·mL^{-1}) 的 220～330 倍。采用 MTT 法对目标化合物进行了体外抗人骨肉瘤细胞 U2OS 活性测试, 结果表明, 所有目标化合物对人骨肉瘤细胞的抑制活性均显著高于大黄酸, 大多数化合物的活性与临床常用抗骨肉瘤药物多柔比星相当, 其中化合物 **4t** 抑制 U2OS 的活性最强, IC_{50} 值为 2.08 μmol·L^{-1}, 略强于多柔比星。体外羟基磷灰石吸附实验表明, 羟基磷灰石对 **4t** 的吸附值为 13.97 μmol·g^{-1}, 高于四环素 (8.24 μmol·g^{-1}), 具有良好的骨靶向性。

关键词: 大黄酸衍生物; 水溶性; 骨亲和性; 抗骨肉瘤活性

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Synthesis and biological evaluation of aminoalcohol rheinate as anti-osteosarcoma agents

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Abstract: Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is the primary anthraquinone in the roots of rhubarb. A recent study showed that rhein can inhibit tumor cell proliferation and induce apoptosis in human tumor cells. However, the clinical application of rhein has been hampered by its poor bioavailability, low aqueous solubility and gastrointestinal disorders. In current study, twenty-four target compounds were designed and synthesized by coupling various hydrophilic alkanolamines to the 2-carboxyl of rhein, and their structures were established by IR, HR-MS, ^{1}H NMR spectra. Solubility test showed that all compounds were 10.04 to 15.08 mg·mL^{-1} in water, which was 220 to 330-fold better than that of rhein (0.045 6 mg·mL^{-1}). All of rhein derivatives displayed more potent anti-tumor activity than rhein, and most of them were comparable to adriamycin, particularly, compound **4t** exhibited IC_{50} value of 2.08 μmol·L^{-1}, more effective than adriamycin (IC_{50} = 2.35 μmol·L^{-1}). Hydroxyapatite adsorption experiment suggests that compound **4t** has a better bone affinity than that of tetracycline.

Key words: rhein derivative; water solubility; bone-affinity; anti-osteosarcoma activity

骨肉瘤 (osteosarcoma, OS) 又称成骨样肉瘤, 是恶性程度较高的骨的原发性肿瘤[1]。以往单纯采用手术治疗, 5 年生存率仅有 20%左右, 辅助化疗的引入使得骨肉瘤患者 5 年生存率可提高到 70%左右。近 30 年来, 尽管手术方式不断改进, 化疗药物不断更新, 但骨肉瘤 5 年生存率并没有得到明显提高, 尤其对复发患者更甚[2−5]。如何有效地降低和控制肺转移率, 提高患者的长期生存率, 化疗占有极其重要的

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地位。

多柔比星、大剂量甲氨蝶呤、顺铂和异环磷酰胺是骨肉瘤化疗中最常用的药物。由于骨组织硬度大，渗透性差，需要大剂量给药，才能在骨组织中达到治疗浓度，加之上述药物均有骨髓抑制、胃肠反应、心脏、肝脏及肾脏毒性等不良反应，药物的治疗指数较低[6−10]。为此，寻找具有骨靶向性的抗骨肉瘤药物很有必要。

大黄酸 (4,5-dihydroxyanthraquinone-2-carboxylic acid, **1**, 合成路线 1) 是传统中药大黄的主要活性成分之一，具有抗炎、抗肿瘤、抗阿尔兹海默病等多种药理活性[11−18]。研究表明，大黄酸可以与 Ca^{2+}形成六元环，具备与骨组织中羟基磷灰石结合的条件，因而具有骨靶向性。近年来的研究表明，将大黄酸与抗肿瘤活性成分偶联，可通过两种药物的协同作用达到提高疗效、降低毒性、靶向给药的目的[19, 20]。大黄酸本身具有一定的抗肿瘤活性，加之毒性低，安全性高，又具骨靶向性，以其为先导化合物研发高效、低毒、具有骨靶向性的大黄酸类抗肿瘤药物具有良好的研发前景。课题组前期曾以藤黄酸和新藤黄酸为先导化合物，合成了系列藤黄酸和新藤黄酸胺基醇酯衍生物，实验结果表明，在藤黄酸和新藤黄酸分子中引入有机胺结构，可以增加化合物的水溶性，提高化合物的抗肿瘤活性[21, 22]。为此，本文在课题组前期工作基础上，以大黄酸为先导化合物，设计、合成了大黄酸胺基醇酯衍生物，以期获得水溶性好，具有高活性和骨靶向性的抗骨肉瘤目标化合物。

以大黄酸为原料，与二溴烷烃发生 *O*-烷基化反应，生成大黄酸溴代烷基酯 (**2a**～**2d**)，再与吗啉、哌啶等有机胺类反应，得到相应的大黄酸胺基醇酯 (**3a**～**3x**)，利用其碱性与盐酸成盐，得到目标化合物 **4a**～**4x** (合成路线 1)，有机胺为甲基哌嗪、乙基哌嗪和羟乙基哌嗪的目标化合物是二元盐，其他为一元盐。

结果与讨论

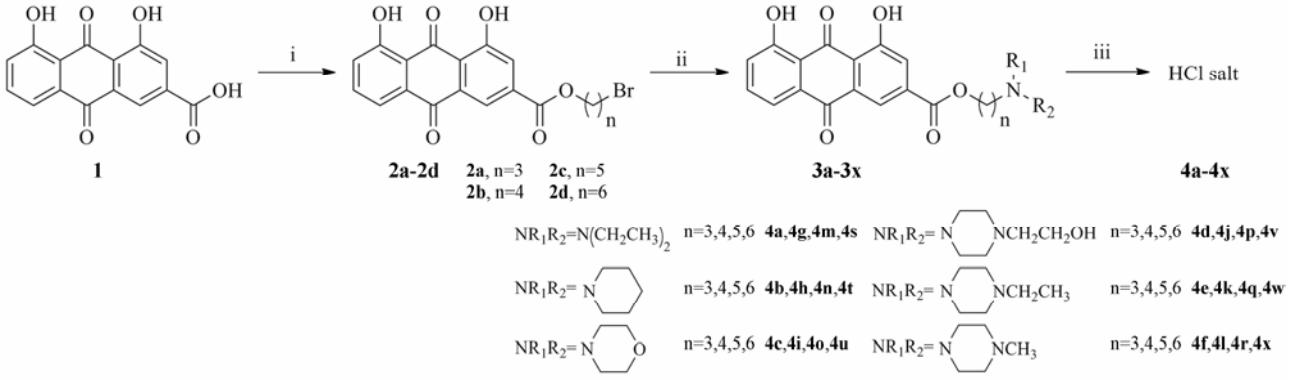
1　化学合成

合成的 4 个中间体 **2a**～**2d** 及 24 个目标化合物的理化常数和波谱数据分别见表 1～4。

Table 1　Physical properties of compounds **2a**−**2d**

Compd.	Character	Yield/%	mp/℃
2a	Yellow powder	87.7	147.2−148.0
2b	Yellow powder	89.2	134.5−135.4
2c	Yellow powder	89.8	130.2−131.6
2d	Yellow powder	91.6	131.4−132.6

大黄酸溶解性能差，本研究分别选用 DMSO、DMF 和 1,4-二氧六环为溶剂进行实验。首先选用碳酸钾为缚酸剂与二溴烷烃反应，结果发现，产物量很少，且后处理操作繁琐；后改用三乙胺为缚酸剂，TLC 检测发现，反应难以进行。这可能与大黄酸在 DMSO、DMF 和 1,4-二氧六环中溶解度小有关。通过查阅文献及反复实验发现，在反应液中添加少量相转移催化剂 TBAB (四正丁基溴化铵)，有助于反应的进行。考虑到 DMSO、DMF、1,4-二氧六环等溶剂沸点较高，后处理操作有一定的难度，尝试用沸点较低的四氢呋喃为反应溶剂。结果表明，反应能较好地进行。在进一步的实验中发现，随着反应物配比的改变，产物收率有较大的差异；反应温度对反应进程和产物的收率也有较大的影响。为此，对反应物的配比及反应温度进行了优化，得到最佳反应条件为：反应物的配比以大黄酸−二溴烷烃=1∶4 (摩尔比)，反应



Scheme 1　Synthetic routes of compounds **4a**−**4x**. Reagents and conditions: (i) Br-$(CH_2)_n$-Br (n = 3−6), THF, TEA, TBAB, rt, 10.5−11.5 h; (ii) Diethylamine, morpholine, piperidine, *N*-methyl piperazine, *N*-ethyl piperazine, or *N*-hydroxyethyl piperazine, K_2CO_3, CH_3CN, 40−50 ℃, 8−9 h; (iii) Isopropanol saturated solution of hydrogen chloride, rt, 12−24 h

Table 2 IR, ^{1}H NMR and MS data of compounds **2a**−**2d**

Compd.	ESI-MS *m*/*z* $[M+H]^+$	IR (KBr) υ/cm	^{1}H NMR (400 MHz, $CDCl_3$)
2a	405.3, 407.2	3 061, 2 920, 1 719, 1 630, 1 474, 1 453, 1 387	11.86 (s, 1H), 11.81 (s, 1H), 8.21−8.19 (m, 1H), 7.73−7.28 (m, 3H), 7.27−7.25 (m, 1H), 4.50 (t, *J* = 6.5 Hz, 2H), 3.57 (t, *J* = 6.4 Hz, 2H), 2.36−2.34 (m, 2H, CH_2)
2b	419.2, 421.4	3 071, 2 956, 1 727, 1 630, 1 470, 1 454, 1 374	12.00 (s, 1H), 11.93 (s, 1H), 8.36 (s, 1H), 7.90 −7.88 (m, 2H), 7.74 (d, *J* = 7.3 Hz, 1H), 7.33−7.31 (m, 1H), 4.44 (t, *J* = 6.4 Hz, 2H), 3.53 (t, *J* = 10.4 Hz, 2H), 2.08−2.05 (m, 4H, CH_2)
2c	433.3, 435.5	3 077, 2 936, 1 723, 1 629, 1 471, 1 454, 1 378	11.95 (s, 1H), 11.90 (s, 1H), 8.33 (s, 1H), 7.94 −7.80 (m, 2H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 4.40 (t, *J* = 6.3 Hz, 2H), 3.48 (t, *J* = 6.4 Hz, 2H), 2.03−1.93 (m, 2H), 1.92−1.82 (m, 2H), 1.72−1.60 (m, 2H)
2d	447.3, 449.2	3 421, 2 947, 1 721, 1 672, 1 630, 1 453, 1 428, 1 399, 1 376	11.98 (s, 1H), 11.92 (s, 1H), 8.35 (s, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.34−7.27 (m, 1H), 4.40 (t, *J* = 6.2 Hz, 2H), 3.44 (t , *J* = 6.8 Hz, 2H), 2.00−1.74 (m, 8H)

Table 3 Physical properties of target compounds **4a**−**4x**

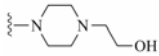
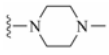
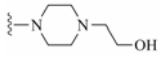
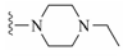
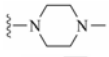
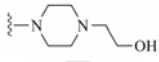
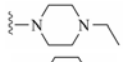
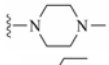
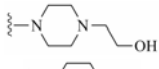
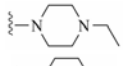
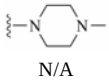
Compd.	NR_1R_2	Character	Yield/%	mp/℃	Solubility/mg·mL^{-1}
4a		Yellow powder	68.2%	>200	13.62
4b		Yellow powder	60.1%	>200	11.32
4c		Yellow powder	58.7%	>200	12.10
4d		Yellow powder	58.1%	>200	12.17
4e		Yellow powder	58.3%	>200	10.05
4f		Yellow powder	58.9%	>200	13.27
4g		Yellow powder	65.6 %	>200	12.98
4h		Yellow powder	59.1%	>200	10.71
4i		Yellow powder	59.2%	>200	11.66
4j		Yellow powder	57.6%	>200	11.96
4k		Yellow powder	57.9%	>200	10.48
4l		Yellow powder	56.8%	>200	14.83
4m		Yellow powder	65.9%	>200	12.43
4n		Yellow powder	64.3%	>200	10.27
4o		Yellow powder	58.8%	>200	12.32
4p		Yellow powder	58.9%	>200	12.21
4q		Yellow powder	57.0%	>200	10.04
4r		Yellow powder	60.3%	>200	13.72
4s		Yellow powder	67.6%	>200	13.84
4t		Yellow powder	56.0%	>200	11.04
4u		Yellow powder	60.2%	>200	12.85
4v		Yellow powder	58.2%	>200	15.08
4w		Yellow powder	60.4%	>200	10.22
4x		Yellow powder	58.5%	>200	12.90
Rhein	N/A	Yellow powder	N/A	>200	0.045 6

Table 4 IR, ^{1}H NMR and MS data of target compounds **4a**−**4x**

Compd.	ESI-HR-MS *m/z* [M+H]$^+$	IR (KBr) *υ*/cm	^{1}H NMR
4a	398.161 5	3 421.8, 2 925.2, 2 675.1, 1 717.0, 1 673.6, 1 638.5, 1 451.1, 1 424.1, 1 267.9	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.33 (s, 1H), 7.89−7.79 (m, 2H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 4.40 (t, *J* = 7.1 Hz, 2H), 2.75 (m, 6H), 2.10−2.05 (m, 2H), 1.16−1.10 (m, 6H)
4b	410.161 7	3 436.3, 3 180.1, 2 959.0, 2 931.0, 2 603.5, 2 475.9, 2 404.3, 1 730.1, 1 679.7, 1 632.1, 1 452.4, 1 415.2	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.23 (s, 1H), 7.79 (s, 1H), 7.74 (d, *J* = 7.4 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 8.9 Hz, 1H), 4.32 (t, *J* = 6.8 Hz, 2H), 2.85−2.81 (m, 6H), 1.80−1.76 (m, 2H), 1.68−1.64 (m, 2H), 1.29−1.24 (m, 4H)
4c	412.141 2	3 436.1, 2 947.0, 2 878.2, 2 418.1, 1 729.3, 1 629.9, 1 453.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 12.05 (s, 1H), 11.98 (s, 1H), 8.41 (d, *J* = 7.1 Hz, 1H), 7.92 (m, 2H), 7.76 (t, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 4.50 (t, *J* = 6.8 Hz, 2H), 3.88−3.85 (m, 4H), 2.72−2.70 (m, 6H), 2.19−2.17 (m, 2H)
4d	455.183 2	3 336.3, 2 984.7, 2 635.5, 2 549.1, 2 429.6, 1 724.9, 1 629.9, 1 475.3, 1 455.1	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.37 (s, 1H), 7.88 (m, 2H), 7.72 (m, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 4.46 (t, *J* = 6.2 Hz, 2H), 3.65 (t, *J* = 6.8 Hz, 2H), 2.62−2.57 (m, 12H), 2.04−2.01 (m, 2H)
4e	439.188 1	3 431.0, 3 183.2, 2 923.8, 2 853.0, 2 651.6, 2 561.9, 2 441.9, 1 725.9, 1 674.7, 1 633.1, 1 469.7, 1 450.5	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 11.99 (s, 1H), 11.92 (s, 1H), 8.16 (s, 1H), 7.88 (m, 2H), 7.77 (m, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 4.45 (t, *J* = 6.4 Hz, 2H), 2.54−2.50 (m, 10H), 2.26−2.23 (m, 2H), 1.30−1.27 (m, 5H)
4f	425.172 6	3 428.7, 3 183.2, 2 558.9, 2 442.1, 1 728.0, 1 631.1, 1 469.7, 1 448.0	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 8.36 (s, 1H), 7.93−7.80 (m, 2H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.31 (m, 1H), 4.45 (t, *J* = 6.6 Hz, 2H), 2.77−2.55 (m, 10H), 2.41 (s, 3H), 2.05−2.01 (m, 2H)
4g	412.175 3	3 422.8, 2 926.3, 2 670.1, 1 719.0, 1 671.6, 1 632.5, 1 450.1, 1 424.1	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.94 (d, *J* = 7.6 Hz, 1H), 7.75 (m, 1H), 7.70−7.50 (m, 2H), 7.40−7.29 (m, 1H), 4.37 (t, *J* = 6.7 Hz, 2H), 3.21−3.18 (m, 6H), 1.89−1.86 (m, 4H), 1.27−1.22 (m, 6H)
4h	424.175 2	3 417.2, 2 923.2, 2 850.7, 1 722.2, 1 629.4, 1 475.6, 1 450.6	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 11.96 (s,1H), 11.92 (s, 1H), 8.14 (s, 1H), 7.92 −7.82 (m, 2H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 4.39 (t, *J* = 7.4 Hz, 2H), 3.48−3.42 (m, 6H), 1.93−1.67 (m, 10H)
4i	426.164 5	3 433.1, 2 943.0, 2 876.2, 2 412.1, 1 727.3, 1 626.9, 1 451.6, 1 296.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.88 (s, 1H), 7.75 (s, 1H), 7.54 (m, 2H), 7.29 (m, 1H), 4.31 (t, *J* = 6.5 Hz, 2H), 3.61 (t, *J* = 6.6 Hz, 4H), 2.50−2.46 (m, 6H), 1.72−1.68 (m, 4H)
4j	469.191 4	3 410.7, 2 935.1, 2 645.4, 2 565.8, 1 719.6, 1 629.6, 1 609.1, 1 452.4, 1 378.7	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.10 (s, 1H), 7.76 (m, 3H), 7.43 (s, 1H), 4.35 (t, *J* = 6.6 Hz, 2H), 3.80 (t, *J* = 6.6 Hz, 2H), 2.55−2.49 (m, 12H), 1.46−1.41 (m, 4H)
4k	453.196 4	3 420.9, 2 975.5, 2 638.6, 2 557.8, 1 718.8, 1 680.8, 1 632.9, 1 451.3	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 8.10 (d, *J* = 7.9 Hz, 1H), 7.88−7.69 (m, 3H), 7.42 (d, *J* = 7.1 Hz, 1H), 4.38 (t, *J* = 6.7 Hz, 2H), 3.11−2.93 (m, 12H), 1.82 (m, 2H), 1.73 (m, 2H), 1.25−1.22 (m, 3H)
4l	439.196 4	3 421.5, 2 934.9, 2 798.1, 1 719.2, 1 673.4, 1 629.7, 1 452.0, 1 418.3, 1 376.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.91 (s, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.61 (s, 2H), 7.34 (d, *J* = 8.1 Hz, 1H), 4.34 (t, *J* = 6.8 Hz, 2H), 2.80−2.39 (m, 15H), 1.95−1.93 (m, 2H)
4m	426.190 0	3 421.3, 2 922.4, 2 650.8, 1 723.2, 1 630.6, 1 476.1, 1 451.3, 1 268.8	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.27 (s, 1H), 7.90−7.76 (m, 2H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 7.1 Hz, 1H), 4.39 (t, *J* = 6.6 Hz, 2H), 3.20 (t, *J* = 7.0 Hz, 4H), 3.08 (t, *J* = 6.0 Hz, 2H), 1.44 (t, *J* = 6.9 Hz, 6H), 1.26−1.22 (m, 6H)
4n	438.185 6	3 422.5, 2 958.0, 1 717.8, 1 631.6, 1 473.8, 1 451.4, 1 378.2	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 12.03 (s, 1H), 11.96 (s, 1H), 8.39 (s, 1H), 7.96 −7.85 (m, 2H), 7.75 (t, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 4.42 (t, *J* = 6.8 Hz, 2H), 2.68−2.24 (m, 6H), 2.32−2.29 (m, 2H), 1.93−1.89 (m, 6H), 1.30−1.26 (m, 4H)
4o	440.164 8	3 421.7, 2 963.8, 2 443.6, 1 724.2, 1 670.4, 1 636.5, 1 452.6, 1 268.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.33 (s, 1H), 7.87 (s, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.70 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 4.39 (t, *J* = 6.6 Hz, 2H), 3.73 (t, *J* = 8.0 Hz, 4H), 2.45−2.41 (m, 6H), 1.91−1.79 (m, 2H), 1.63−1.61 (m, 2H), 1.52−1.49 (m, 2H)
4p	483.207 0	3 393.1, 2 948.2, 1 721.6, 1 630.9, 1 452.1, 1 276.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.28 (s, 1H), 7.89−7.75 (m, 2H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 9.2 Hz, 1H), 4.36 (t, *J* = 6.2 Hz, 2H), 3.63 (t, *J* = 6.7 Hz, 2H), 2.77−2.32 (m, 12H), 1.84−1.80 (m, 2H), 1.63−1.61 (m, 2H), 1.49−1.47 (m, 2H)
4q	467.216 1	3 420.6, 2 974.6, 2 441.7, 1 721.6, 1 673.1, 1 626.5, 1 452.7, 1 380.3	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.31 (s, 1H), 7.90−7.59 (m, 3H), 7.29 (s, 1H), 4.37 (t, *J* = 6.5 Hz, 2H), 2.97−2.45 (m, 12H), 1.83 (s, 2H), 1.58−1.54 (m, 4H), 1.20 (s, 3H)
4r	453.200 7	3 433.1, 2 950.6, 2 641.9, 2 545.9, 1 730.2, 1 673.7, 1 625.3, 1 451.3	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 11.95 (s, 1H), 11.91(s, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.88−7.80 (m, 2H), 7.75 (d, *J* = 6.6 Hz, 1H), 7.44 (d, *J* = 7.4 Hz, 1H), 4.38 (t, *J* = 6.4 Hz, 2H), 3.57−1.53 (m, 10H), 3.05−3.01 (m, 3H), 1.70−1.68 (m, 2H), 1.47−1.42 (m, 4H)
4s	440.205 2	3 426.3, 2 937.1, 2 668.8, 1 720.2, 1 672.3, 1 632.0, 1 569.3, 1 473.3, 1 451.3, 1 408.2	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.90 (s, 1H), 7.78 (s, 1H), 7.60 (s, 2H), 7.34 (d, *J* = 7.4 Hz, 1H), 4.31 (t, *J* = 6.2 Hz, 2H), 3.16−3.07 (m, 6H), 1.76−1.73 (m, 4H), 1.48−1.44 (m, 4H), 1.25−1.20 (m, 6H)
4t	452.205 2	3 421.1, 2 920.6, 2 843.7, 1 722.4, 1 626.3, 1 472.6, 1 456.9	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.20 (s, 1H), 7.78−7.67 (m, 2H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 8.3 Hz, 1H), 4.28 (t, *J* = 6.3 Hz, 2H), 2.95−2.92 (m, 6H), 1.90−1.86 (m, 2H), 1.77−1.74 (m, 2H), 1.45−1.40 (m, 4H)
4u	454.184 4	3 433.1, 2 935.9, 2 866.1, 1 727.1, 1 626.3, 1 473.8, 1 455.6	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.41 (s, 1H), 7.91 (m, 2H), 7.74 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 4.40 (t, *J* = 6.5 Hz, 2H), 3.82−3.79 (s, 4H), 2.55−2.51 (m, 6H), 1.90−1.80 (m, 2H), 1.53−1.49 (m, 6H)
4v	497.226 7	3 442.7, 3 314.7, 2 922.7, 2 575.2, 1 720.8, 1 672.8, 1 631.2, 1 457.8, 1 377.0	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.36 (s, 1H), 7.87 (m, 2H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.31 (m, 1H), 4.38 (t, *J* = 6.4 Hz, 2H), 3.72−3.70 (m, 2H), 2.91−2.56 (m, 12H), 1.84−1.80 (m, 2H), 1.63−1.61 (m, 2H), 1.50−1.48 (m, 2H), 1.45−1.42 (m, 2H)
4w	481.231 6	3 426.3, 3 080.7, 2 936.4, 2 810.0, 1 718.9, 1 674.1, 1 629.2	^{1}H NMR (400 MHz, DMSO-d_6) *δ* 8.34 (s, 1H), 7.91−7.78 (m, 2H), 7.70 (t, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 4.37 (t, *J* = 6.7 Hz, 2H), 2.69−2.38 (m, 12H), 1.83−1.81 (m, 2H), 1.59−1.39 (m, 6H), 1.12 (t, *J* = 7.0 Hz, 3H)
4x	467.216 3	3 423.1, 2 929.4, 2 852.8, 2 766.7, 2 359.6, 1 721.0, 1 676.5, 1 626.7, 1 471.7, 1 416.1, 1 268.1	^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.32 (s, 1H), 7.90−7.76 (m, 2H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 6.5 Hz, 1H), 4.36 (t, *J* = 6.4 Hz, 2H), 3.24−2.18 (m, 13H), 1.81−1.78 (m, 2H), 1.66−1.62 (m, 2H), 1.54−1.37 (m, 4H)

温度为 20～30 ℃。

在制备大黄酸胺基醇酯基酯 (**3a**～**3x**) 的过程中, 随着反应物配比的改变, 产物收率有较大的差异; 反应温度对反应进程和产物的收率也有较大的影响。实验表明, 当大黄酸溴代烷基酯−有机胺=1∶4 (摩尔比), 反应温度为 40～50 ℃时, 反应收率较高, 可达 69% 以上。

2 目标化合物的水溶性

本文对大黄酸进行修饰的主要目的之一是提高水溶性, 便于临床使用, 故测试了目标化合物和大黄酸在水中的溶解度。表 3 实验数据显示, 大黄酸氨基醇酯类化合物的水溶性与大黄酸相比有较大幅度地增加, 目标化合物在水中的溶解度为 10.04～15.08 $mg\cdot mL^{-1}$, 是大黄酸 (溶解度为 0.045 6 $mg\cdot mL^{-1}$) 的 220～330 倍。

3 细胞毒活性

以大黄酸和多柔比星为阳性对照, 采用 MTT 法测试了目标化合物对人骨肉瘤细胞 U2OS 的体外抗细胞增殖活性。实验结果见表 5。

由表 5 可知, 所合成的大黄酸胺基醇酯类化合物能够不同程度地抑制人骨肉瘤细胞 U2OS 的增殖。其中, 化合物 **4c**、**4h**、**4i**、**4o** 和 **4u** 对人骨肉瘤增殖的抑制活性较弱, 其 IC_{50} 值大于 20 $\mu mol\cdot L^{-1}$; 其他化合物与阳性对照药多柔比星相当, 具有较强的抑制人骨肉瘤细胞增殖作用, 以 **4t** 的活性为最强, IC_{50} 值为 2.08 $\mu mol\cdot L^{-1}$, 略优于多柔比星。

4 羟基磷灰石对化合物 4t 的吸附作用

采用羟基磷灰石吸附实验测定了化合物 **4t** 的体外骨亲和性, 实验数据显示, 羟基磷灰石对 **4t** 的吸附值为 13.97±0.13 $\mu mol\cdot g^{-1}$, 高于对四环素 (8.24±0.12 $\mu mol\cdot g^{-1}$) 和大黄酸 (6.85±0.08 $\mu mol\cdot g^{-1}$) 的吸附值, 表明 **4t** 具有良好的骨亲和性。

5 构效关系初步分析

由活性测试结果可以看出, 目标化合物的抗骨肉瘤细胞活性与连接臂碳数及不同的有机胺相关: 当有机胺片段相同时, 连接臂碳数不同, 其活性也有所不同, 碳数为 6 时抗肿瘤活性较好, 如 **4t** > **4n** > **4b** > **4h**, **4v** > **4j** > **4p** > **4d**, 但对目标化合物的活性影响不明显; 不同的有机胺对目标化合物的活性影响各不相同, 当分子中引入吗啉环, 得到的目标化合物其抗骨肉瘤活性较差, IC_{50} 值均高于 20 $\mu mol\cdot L^{-1}$, 而当分子中引入乙基哌嗪时, 所得化合物的 IC_{50} 值均为较低的个位数, 呈现出较好的抗骨肉瘤活性; 在活性最好的化合物 **4t** 中, 胺基为哌啶, 连接臂碳数为 6, 而在化合物 **4h** 中, 有机胺部分同为哌啶, 连接臂碳数为 3, 其 IC_{50} 值高于 20 $\mu mol\cdot L^{-1}$, 抗骨肉瘤活性较差。

Table 5 Effects of the target compounds **4a**−**4x** on proliferation of U2OS

Compd.	*In vitro* cytotoxicity (IC_{50}/$\mu mol\cdot L^{-1}$)
	U2OS
4a	7.44 ± 0.23
4b	6.89 ± 0.31
4c	> 20
4d	7.02 ± 0.16
4e	3.42 ± 0.15
4f	5.47 ± 0.28
4g	2.67 ± 0.08
4h	> 20
4i	> 20
4j	2.34 ± 0.11
4k	3.23 ± 0.06
4l	3.75 ± 0.12
4m	5.39 ± 0.31
4n	3.55 ± 0.18
4o	> 20
4p	2.84 ± 0.09
4q	2.85 ± 0.05
4r	2.68 ± 0.15
4s	3.13 ± 0.22
4t	2.08 ± 0.07
4u	> 20
4v	2.28 ± 0.06
4w	5.94 ± 0.21
4x	3.19 ± 0.19
Adriamycin	2.25 ± 0.28
Rhein	> 100

6 小结

水溶性测试实验表明, 目标化合物的水溶性均有大幅提高 (溶解度为 10.04～15.08 $mg\cdot mL^{-1}$), 是大黄酸溶解度 (0.045 6 $mg\cdot mL^{-1}$) 的 220～330 倍。体外采用 MTT 法对所有化合物进行抗骨肉瘤 U2OS 细胞活性筛选, 结果表明, 所有目标化合物对骨肉瘤细胞的抑制活性显著高于大黄酸, 大多数化合物的活性与多柔比星相当, 其中, 化合物 **4t** 活性最强, IC_{50} 值为 2.08 $\mu mol\cdot L^{-1}$。体外羟基磷灰石吸附实验表明, **4t** 的骨亲和性强于四环素。

实验部分

LCQ ADVANTAGE MAX 液质联用质谱仪 (美国 FINNIGAN 公司); Nicolet Acatar 370 DTGS 型红外光谱仪 (美国 Thermo Electron 公司); AV400 型核磁共振仪 (德国 Bruker 公司, TMS 为内标); 薄层硅胶 G

板 (合肥森瑞有限公司, 100 mm×100 mm); 大黄酸 (西安小草植物科技有限责任公司, 含量大于 98%), 试剂为市售分析纯。

1 合成部分

1.1 中间体 2a～2d 的合成通法 将大黄酸 (284 mg, 1 mmol)、三乙胺 (4 mmol)、四正丁基溴化铵 (322 mg, 1 mmol) 和 THF 10 mL 加入到 50 mL 圆底烧瓶中, 室温搅拌 5 min 后, 加入二溴烷烃 (4 mmol), 室温搅拌, TLC 监测反应进程。反应完毕, 过滤, 滤液减压浓缩, 硅胶柱色谱纯化 (乙酸乙酯−石油醚=1∶5) 得中间体大黄酸溴代烷基酯。

1.2 目标化合物 4a～4x 将 **2a～2d** (0.5 mmol)、K_2CO_3 (276 mg, 2.0 mmol)、KI (83 mg, 0.50 mmol) 和含氮杂环 (4 mmol) (二乙胺、哌啶、吗啉、*N*-甲基哌嗪、*N*-乙基哌嗪、*N*-羟乙基哌嗪) 置于 100 mL 圆底烧瓶中, 加乙腈 10 mL, TLC 监测反应进程, 40～50 ℃反应 8～9 h。将反应液降到室温, 加水 50 mL, 用稀盐酸调 pH 至 7, 用 CH_2Cl_2 (30 mL×3) 萃取, 合并有机层后用饱和食盐水洗涤 3 次, 无水硫酸钠干燥, 浓缩, 柱色谱纯化 (甲醇−二氯甲烷=1∶10, *v*/*v*), 得红褐色蜡状物, 加氯化氢的异丙醇饱和溶液 5 mL, 室温搅拌过夜, 过滤, 烘干, 得黄色粉末。

2 化合物 4a～4x 水溶性测试

精密称取 **4a～4x**, 用蒸馏水定容至 100 mL, 分别移取 0.5、1、1.5、2、2.5 mL 定容至 10 mL 中, 平行测其吸光度 3 次。计算线性回归方程。取适量过量的 **4a～4x** 加入一定量水中, 37 ℃恒温摇床 72 h, 离心取上清液, 紫外分光光度法检测其含量, 计算出溶解度。

3 细胞毒活性测试

实验选择人骨肉瘤细胞株 U2OS 测试受试目标化合物和阳性对照大黄酸、多柔比星对受试细胞的抑制率。细胞在 37 ℃、5% CO_2 饱和湿度的培养箱中常规培养。培养液为含 10% 热灭活胎牛血清、青霉素 100 u·mL^{-1} 和链霉素 100 u·mL^{-1} 的 RPMI 1640 或 DMEM 细胞培养基, 48 h 更换培养液, 细胞汇合时, 用 0.25% 胰蛋白酶消化传代。实验用细胞均处于对数生长期, 台盼蓝拒染法表明细胞活力>95%。

取处于对数生长期状态良好的细胞一瓶, 加入消化液 (0.125% 胰蛋白酶 + 0.01% EDTA) 消化, 计数每毫升 2×10^4～4×10^4 个, 制成细胞悬液接种于 96 孔板上, 每孔 180 μL, 置恒温 CO_2 培养箱中培养 24 h。换液, 加入受试药物, 每孔 20 μL, 培养 48 h。将 MTT 加入 96 孔板中, 每孔 20 μL, 培养箱中孵育 4 h。吸去上清液, 加 DMSO, 每孔 150 μL, 平板摇床上振摇 10 min。受试物考察 7 个浓度 (0.1～10 μmol·L^{-1}), 用酶联免疫监测仪在波长为 570 nm 处测定每孔的吸光度, 分别计算各浓度下的细胞抑制率。

抑制率计算方法:

$$细胞抑制率=\frac{阴性对照孔相对OD值-药敏孔相对OD值}{阴性对照孔相对OD值}\times100\%$$

阴性对照孔相对 OD 值 = 阴性对照孔绝对 OD 值 − 空白对照孔绝对 OD 值

药敏孔相对 OD 值 = 药敏孔绝对 OD 值 − 空白对照孔绝对 OD 值

应用 SPSS17.0 通过几率单位加权回归法 (Bliss 法) 计算 IC_{50}。

4 化合物 4t 体外羟基磷灰石吸附实验

精密配制四环素和待测品的浓度分别为 200、100、50、20、10 μmol·L^{-1} 的无水乙醇溶液, 用紫外分光光度计分别测定其吸光度值, 得出吸光度值与浓度之间的线性关系。精确移取 200 μmol·L^{-1} 待测液 5 mL 至 10 mL 量瓶中, 定容。每一个样品共取 4 份该溶液, 分别向其中的 3 份加入羟基磷灰石 25 mg, 另一份作为空白对照。各样品在超声波振荡器中振荡 1 min 后, 置于暗处室温平衡 16 h。过滤得到澄清透明的溶液, 空白和滤液用分光光度计测定其吸光度值, 计算出平衡后样品的摩尔浓度。利用公式 $A = V\cdot\Delta C/m$ 计算出各化合物对羟基磷灰石的吸附值, 式中: V 为溶液的体积 (L); ΔC 为各样品溶液与羟基磷灰石平衡前后的浓度差 (μmol·L^{-1}); m 为羟基磷灰石的质量 (g)。再计算出吸附率, 即吸附在羟基磷灰石上的待测化合物占吸附前溶液中待测物总量的百分比[23]。

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