3-芳苄叉基-氟喹啉-4-酮衍生物的设计、合成与抗肿瘤活性

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摘要: 为发现氟喹诺酮有效结构修饰方法以提高其抗肿瘤活性, 基于片段药物分子设计策略, 芳苄叉基作为C-3羧基的生物电子排体, 并与4-羰基共同构建*α*,*β*-不饱和酮骨架, 进而设计合成了1-环丙基-6-氟-7-(4-甲基哌嗪-1-基)-3-芳苄叉基-喹啉-4(1*H*)-酮 (**4a**～**4p**) 目标化合物, 其结构经元素分析和光谱数据确证。体外抗肿瘤实验结果表明, 目标化合物对Hep-3B、Capan-1和HL60三种实验癌细胞株的活性显著高于母体环丙沙星, 其中, 卤代苯基或芳香杂环基化合物的活性强于其他取代的活性, 尤其是氯苯基化合物 (**4j**、**4k**) 对Capan-1的IC_{50}与对照药多柔比星相当。为此, 芳叉苄基替代C-3羧基所构建的氟喹诺酮不饱和酮有利于提高其抗肿瘤活性, 提示*α*, *β*-不饱和酮结构或其片段作为C-3羧基的潜在等排体有待进一步发展。

关键词: 氟喹诺酮; 二氢喹啉酮; 不饱和酮; 生物电子等排体; 抗肿瘤活性

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Design, synthesis and antitumor activity of 3-arylidene-4-fluoroquinolin-4-ones as ciprofloxacin derivatives

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Abstract: To identify an effective structural modification strategy for improving the antitumor activity of fluoroquinolones, sixteen new 1-cyclopropyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-3-arylidene-2,3-dihydroquinolin-4(1*H*)-ones compounds (**4a**−**4p**), were designed and synthesized by a condensation reaction of dihydroquinolin-4-one (**3**) and aromatic aldehydes, based on the structure of ciprofloxacin (**1**). Their structures were characterized by elemental analysis and spectral data, and anti-cell proliferative activities against Hep-3B, Capan-1 and HL60 cell lines were measured by an MTT assay. Preliminary pharmacological results indicated that the synthesized target compounds had greater potency than ciprofloxacin (**1**). SAR revealed that the halophenyl compounds such as fluorophenyl (**4h**, **4i**), chlorophenyl (**4j**, **4k**) or bromophenyl compounds (**4l**, **4m**) and aromatic heterocyclic compounds such as furanly (**4n**) or pyridyl compounds (**4o**, **4p**) demonstrated better activity than the control compounds, and the IC_{50} values of the chlorophenyl compounds **4j** and **4k** against Capan-1 cell growth were comparable to that of doxorubicin. Thus, a 3-arylidene as an isostere of the C-3 carboxylic acid group appears to be beneficial in improving the antitumor activity of fluoroquinolone. Furthermore, an *α*,*β*-unsaturated ketone fragment

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used as a potential bioisostere of C-3 carboxylic acid group may warrant further study.

Key words: fluoroquinolone; dihydroquinolinone; unsaturated ketone; bioisostere; antitumor activity

新药研发是一项高投入、高风险、高耗时的复杂智力创新过程, 而基于结构片段药物设计原理, 利用现有药物的优势药效团进行拼合或生物等排或骨架迁越等药物化学技术构建结构多样的化合物库, 并通过活性筛选发现苗头化合物是新药研发最经济有效的策略[1-3]。其中, 最具优势特色的*α*,*β*-不饱和酮骨架不仅作为重要的活性有机合成子参与构建结构多样的功能有机化合物, 同时也是天然有效成分查尔酮[4,5]和黄酮类[6]及甾体激素如雄性激素[7]、孕激素[8]、肾上腺皮质激素类[9]及利尿药依他尼酸[10]和靶向酪氨酸激酶抑制剂舒尼替尼[11]等临床药物的特征药效团骨架。除此外, 因*α*,*β*-不饱和酮片段可与大分子配体发生络合效应及迈克尔加成反应而产生广泛的药理活性已成为多种蛋白激酶类药物的重要共价修饰基备受关注[12,13]。另外, 在众多的药物作用靶点中, 拓扑异构酶 (topo) 不仅是氟喹诺酮药物的抗菌作用靶点[14], 也是抗肿瘤药物的重要作用靶标, 且有多个拓扑异构酶抑制剂有效应用于肿瘤临床治疗[15]。然而, 临床拓扑异构酶抑制剂因存在水溶性差和体内代谢不稳定而导致其生物利用度低及选择性低而导致不良反应大等亟待要克服的局限性, 为此, 基于氟喹诺酮的结构特征及作用机制, 试图通过药物化学结构修饰方法将氟喹诺酮的抗菌活性转为抗肿瘤活性, 进一步拓展拓扑异构酶抑制剂及氟喹诺酮研究的新领域[16]。研究发现, C-3 羧基杂环等排体用*α*,*β*-不饱和酮骨架作为修饰基而构建的C-3稠杂环*α*,*β*-不饱和酮类 (图 1A)[17]、绕丹宁不饱和酮酰胺类 (图 1B)[18]及噻唑不饱和酮类 (图 1C)[19]可显著改善其抗肿瘤活性, 由此推测*α*,*β*-不饱和酮结构可能是发挥抗肿瘤活性的重要药效团片段。受*α*,*β*-不饱和酮骨架在构建药理活性分子重要性的启发, 本文试图保留已知化合物**A**、**B**、**C**的C-3取代基相同的*α*,*β*-不饱和酮结构片段, 即将芳苄叉基部分进行迁越至氟喹诺酮骨架3-位, 并与C-4羰基共同构建新的*α*,*β*-不饱和酮结构, 进而设计了氟喹诺酮C-3芳苄叉基不饱和酮类目标化合物 (**4**) (图 1)。

目标化合物 (**4a**～**4p**) 的制备见合成路线 1 所示。市售环丙沙星 (**1**) 经 Eschweiler-Clarke 甲基化反应生成*N*-甲基环丙沙星 (**2**), 在甲醇中与硼氢化钠发生还原和脱羧反应得到2,3-二氢喹啉-4(1*H*)-酮中间体 (**3**), 然后与芳香醛发生 Claisen-Schmidt 缩合反应形成C-3芳苄叉基而构建氟喹诺酮不饱和酮衍生物 (**4a**～**4p**)。

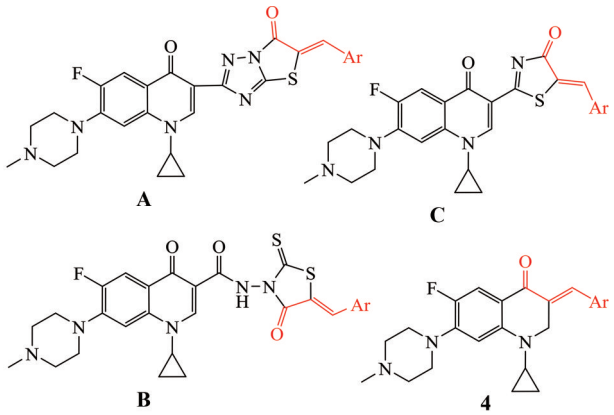
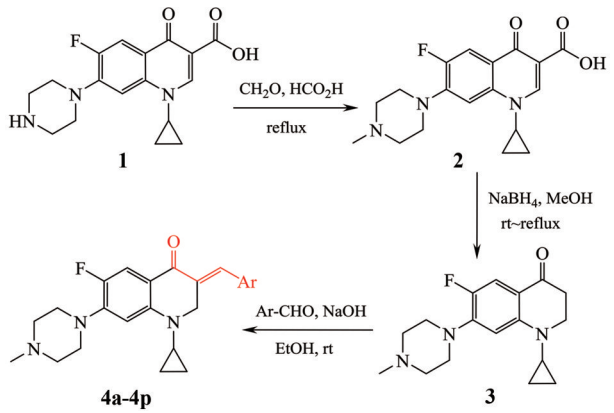


Figure 1 From the known compounds (**A**, **B**, **C**) to the target compounds **4**



Ar: C_6H_5 (**a**); 4-MeO-C_6H_4 (**b**); 2-MeO-C_6H_4 (**c**); 4-Me-C_6H_4 (**d**); 3-Me-C_6H_4 (**e**); 3,4-OCH_2O-C_6H_3 (**f**); 3,4,5-$(MeO)_3$-C_6H_2 (**g**); 4-F-C_6H_4 (**h**); 3-F-C_6H_4 (**i**); 4-Cl-C_6H_4 (**j**); 3-Cl-C_6H_4 (**k**); 4-Br-C_6H_4 (**l**); 3-Br-C_6H_4 (**m**); 2-furyl (**n**); 3-pyridyl (**o**); 4-pyridyl (**p**).

Scheme 1 Synthetic route of the target compounds **4a**–**4p** from ciprofloxacin **1**

结果与讨论

1 化学部分

氟喹诺酮**2**骨架中的2, 3-双键受其3-羧基和4-羰基吸电子效应的影响而表现出高度的缺电子性, 易与硼氢化钠释放出的活性氢负离子 (H^-) 发生加成反应而被还原为易脱羧基的*β*-酮酸型结构, 接着发生脱羧基反应到中间体**3**。实验中发现, 如果加大硼氢化钠的用量, 会导致C-4羰基进一步被还原到四氢喹啉-4-醇副产物的生成。同时, 如果长时间回流反应, 副产物四氢喹啉-4-醇的羟基将发生消去反应转化为1,2-二氢喹啉新副产物。因此, 硼氢化钠的用量、反应温度和反应时间等因素的控制对**3**的产率及纯度极为重要。中

间体**3**与芳香醛在碱性催化下易发生经典的Claisen-Schmidt缩合反应形成C-3芳苄叉基。中间体**3**的^{1}H NMR仅在低场δ 7.20和6.20处出现对应C-5和C-8的2个单质子双峰、高场δ 3.50和2.50处出现对应C-2和C-3的2个双质子多峰, 而在更低场δ 9.0、15.0处均未出现**2**所对应的C-2和3-COOH单质子单峰, 表明**3**不存在**2**的2,3-双键, 而**2**被还原脱羧生成了预期产物**3**。目标物**4**与**3**的^{1}H NMR相比较, **4**在低场δ 3.60处出现双质子单峰化学位移, 而未出现**3**的δ 2.50处双质子多峰化学位移峰, 同时**4**除典型的芳基化学位移峰外, 而在δ 8.0处出现特征单质子单峰可归属3-叉基(=CH)质子, 表明化合物**4**中出现3-芳苄叉基, 同时C-5和C-8的相应的单质子双峰向低场移动约0.50 ppm。另外, 虽然元素分析值及MS测定值也与目标化合物**4**的结构组成相一致, 但所得到产物的3-叉基双键是否移位到环内形成3-苄基-喹啉-4-酮结构仍需要进一步确证。中间体**3**和目标化合物**4**的结构经^{1}H NMR、MS及元素分析确证, 其收率、物理常数及波谱数据见表1、2。

2 抗肿瘤构效关系

初步的体外抗肿瘤实验结果(表3)表明, 合成的16个新目标化合物(**4a**～**4p**)对Hep-3B、Capan-1及HL60三种实验癌细胞的IC_{50} < 40.0 μmol·L^{-1}, 而母体环丙沙星**1**的IC_{50} > 100 μmol·L^{-1}, 表明目标物的抗肿瘤活性强于母体, 提示C-3羧基并非是抗肿瘤活性所必需的药效团结构, 而用芳苄叉基替代C-3羧基构建的3-芳苄叉基-喹啉-4-酮类有利于提高抗肿瘤活性。然而, 目标物的IC_{50}均大于对照多柔比星, 但与前期研究工作的C-3杂环不饱和酮类化合物的IC_{50}相比较, 所对应芳苄叉基相同的化合物其IC_{50}并没有显著的差异, 为此, 进一步表明芳苄叉基杂环酮替代C-3羧基并非必要, 仅用芳苄叉基部分替代C-3羧基也可提高氟喹诺酮的抗肿瘤活性, 由此推测α, β-不饱和酮结构片段作为C-3羧基的取代基将对其抗肿瘤活性产生积极的影响。与此同时, 初步的构效关系表明, 参与构建芳苄叉基的芳基为供电子的甲氧基苯基时, 随着甲氧基个数的增加或体积的增大而导致IC_{50}增大, 不利于提高抗肿瘤活性, 如三甲氧基化合物(**4g**)对3种实验癌细胞的IC_{50}均最大, 表现出较低的活性; 同时, 虽然F、Cl、Br原子半径及电负性有迥然差异, 而卤苯基或芳香杂环类的活性均强于其他取代基化合物, 尤其是氯苯基化合物(**4j**、**4k**)对Capan-1的活性与抗肿瘤药多柔比星相当。总之, 芳苄叉基的空间效应、电效应对活性的影响是综合性因素所决定的, 选择适当的芳基取代基进一步提高氟喹诺酮不饱和酮的抗肿瘤活性仍值得探索和发现。

3 结论

基于氟喹诺酮的作用机制及其结构特征, 用芳苄叉基替代C-3羧基所构建的新氟喹诺酮不饱和酮衍生物以及已知C-3芳苄叉杂环酮类化合物的抗肿瘤活性高于母体环丙沙星, 表明氟喹诺酮的C-3羧基及其杂环不饱和酮等排体并非是抗肿瘤活性所必需的药效团结构, 而以α,β-不饱酮结构片段作为C-3羧基的等排体构建新型的抗肿瘤氟喹诺酮不饱和酮类化合物值得关注。

实验部分

熔点用WK-1B数字熔点仪(上海精密科学仪器厂), 毛细管法, 温度未校正; AM-400型核磁共振仪(德国Bruker公司), DMSO-d_6为溶剂; Esquire LC型质谱

Table 1 Physical constants and spectral data of intermediates **3** and target compounds (**4a**−**4p**)

Compd.	Yield/%	mp/℃	Elemental analysis (%, Calcd.)			MS (*m/z*)
			C	H	N	$[M+H]^+$ (Calcd.)
4	61.7	132−134	67.54 (67.30)	7.10 (7.31)	14.07 (13.85)	304 (303.38)
4a	70.2	186−188	73.86 (73.63)	6.50 (6.69)	10.95 (10.73)	392 (391.49)
4b	76.6	191−193	71.45 (71.24)	6.55 (6.70)	10.18 (9.97)	422(421.52)
4c	61.8	173−175	71.48 (71.24)	6.58 (6.70)	10.20 (9.97)	422 (421.52)
4d	72.3	166−168	74.28 (74.05)	6.78 (6.96)	10.57 (10.36)	406 (405.52)
4e	65.4	162−164	74.30 (74.05)	6.82 (6.96)	10.62 (10.36)	406 (405.52)
4f	82.7	203−205	69.17 (68.95)	5.85 (6.02)	9.80 (9.65)	436 (435.50)
4g	64.2	187−189	67.56 (67.34)	6.56 (6.70)	8.95 (8.73)	482 (481.57)
4h	78.3	203−205	70.64 (70.40)	6.36 (6.15)	10.53 (10.26)	410 (409.48)
4i	64.8	185−187	70.60 (70.40)	5.89 (6.15)	10.48 (10.26)	410 (409.48)
4j	76.5	194−195	67.92 (67.68)	5.78 (5.92)	10.06 (9.87)	426 (425.94)
4k	63.8	182−184	67.90 (67.68)	5.74 (5.92)	10.12 (9.87)	426 (425.94)
4l	73.5	207−209	61.53 (61.28)	5.18 (5.36)	9.17 (8.93)	470 (470.39)
4m	68.3	212−214	61.54 (61.28)	5.22 (5.36)	9.18 (8.93)	470 (470.39)
4n	65.3	210−212	66.72 (66.47)	6.26 (6.09)	10.82 (10.57)	398 (397.52)
4o	72.6	223−225	70.64 (70.39)	6.57 (6.42)	14.53 (14.28)	393 (392.48)
4p	76.4	226−228	70.62 (70.39)	6.27 (6.42)	14.54 (14.28)	393 (392.48)

Table 2 ^{1}H NMR data of intermediates **3** and target compounds (**4a**–**4p**)

Compd.	^{1}H NMR (400 MHz, DMSO-d_6)
3	0.93–1.20 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, N-CH_3), 2.48–2.53 (m, 2H, 3-H), 2.82 (t, *J* = 6.4 Hz, 4H, piperazine-H), 3.10 (t, *J* = 6.4 Hz, 4H, piperazine-H), 3.50–3.68 (m, 3H, 2-H and *c*-Pr-H), 6.15 (d, *J* = 7.6 Hz, 1H, 8-H), 7.26 (d, *J* = 13.6 Hz, 1H, 5-H)
4a	1.00–1.21 (m, 4H, *c*-Pr-H), 2.21 (s, 3H, CH_3), 2.90–3.15 (m, 8H, piperazine-H), 3.50–3.72 (m, 3H, 2-H and *c*-Pr-H), 7.11–7.32 (m, 6H, Ph-H and 8-H), 7.64 (d, *J* = 13.6 Hz, 1H, 5-H), 7.92 (s, 1H, 3-=CH)
4b	1.00–1.21 (m, 4H, *c*-Pr-H), 2.22 (s, 3H, CH_3), 2.92–3.17 (m, 8H, piperazine-H), 3.50–3.71 (m, 6H, 2-H, *c*-Pr-H and OCH_3), 6.79–7.35 (m, 5H, Ph-H and 8-H), 7.68 (d, *J* = 13.6 Hz, 1H, 5-H), 7.88 (s, 1H, 3-=CH)
4c	1.02–1.21 (m, 4H, *c*-Pr-H), 2.21 (s, 3H, CH_3), 2.93–3.16 (m, 8H, piperazine-H), 3.52–3.76 (m, 6H, 2-H, *c*-Pr-H and OCH_3), 6.76–7.35 (m, 5H, Ph-H and 8-H), 7.66 (d, *J* = 13.6 Hz, 1H, 5-H), 7.87 (s, 1H, 3-=CH)
4d	0.96–1.20 (m, 4H, *c*-Pr-H), 2.21, 2.35 (2s, 6H, 2×CH_3), 2.90–3.15 (m, 8H, piperazine-H), 3.51–3.76 (m, 3H, 2-H and *c*-Pr-H), 6.75–7.50 (m, 5H, Ph-H and 8-H), 7.64 (d, *J* = 13.6 Hz, 1H, 5-H), 7.86 (s, 1H, 3-=CH)
7e	0.98–1.20 (m, 4H, *c*-Pr-H), 2.21, 2.36 (2s, 6H, 2×CH_3), 2.93–3.18 (m, 8H, piperazine-H), 3.51–3.78 (m, 3H, 2-H and *c*-Pr-H), 6.78–7.26 (m, 5H, Ph-H and 8-H), 7.68 (d, *J* = 13.6 Hz, 1H, 5-H), 7.88 (s, 1H, 3-=CH)
4f	1.00–1.23 (m, 4H, *c*-Pr-H), 2.22 (s, 3H, CH_3), 3.12–3.64 (m, 11H, piperazine-H, 2-H and *c*-Pr-H), 5.92 (s, 2H, OCH_2O), 6.77–7.38 (m, 5H, Ph-H and 8-H), 7.73(d, *J* = 13.6 Hz, 1H, 5-H), 7.92 (s, 1H, 3-=CH)
4g	0.93–1.22 (m, 4H, *c*-Pr-H), 2.22 (s, 3H, CH_3), 3.00–3.14 (m, 8H, piperazine-H), 3.50–3.72 (m, 12H, 2-H, *c*-Pr-H and 3×OCH_3), 6.68 (s, 2H, Ph-H), 7.36 (d, *J* = 7.6 Hz, 1H, 5-H), 7.68 (d, *J* = 13.6 Hz, 1H, 5-H), 7.85 (s, 1H, 3-=CH)
4h	1.01–1.22 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.05–3.24 (m, 8H, piperazine-H), 3.51–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.20–7.38 (m, 5H, Ph-H and 8-H), 7.70 (d, *J* = 13.6 Hz, 1H, 5-H), 8.00 (s, 1H, 3-=CH)
4i	1.00–1.22 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.06–3.25 (m, 8H, piperazine-H), 3.53–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.23–7.40 (m, 5H, Ph-H and 8-H), 7.72 (d, *J* = 13.6 Hz, 1H, 5-H), 8.02 (s, 1H, 3-=CH)
4j	1.01–1.23 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.08–3.26 (m, 8H, piperazine-H), 3.51–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.02–7.38 (m, 5H, Ph-H and 8-H), 7.71 (d, *J* = 13.6 Hz, 1H, 5-H), 8.00 (s, 1H, 3-=CH)
4k	0.92–1.21 (m, 4H, *c*-Pr-H), 2.21 (s, 3H, CH_3), 3.05–3.26 (m, 8H, piperazine-H), 3.51–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.22–7.38 (m, 5H, Ph-H and 8-H), 7.71 (d, *J* = 13.6 Hz, 1H, 5-H), 7.95 (s, 1H, 3-=CH)
4l	1.00–1.22 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.12–3.27 (m, 8H, piperazine-H), 3.53–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.05–7.37 (m, 5H, Ph-H and 8-H), 7.71 (d, *J* = 13.6 Hz, 1H, 5-H), 8.01 (s, 1H, 3-=CH)
4m	0.98–1.20 (m, 4H, *c*-Pr-H), 2.22 (s, 3H, CH_3), 3.07–3.26 (m, 8H, piperazine-H), 3.51–3.71 (m, 3H, 2-H and *c*-Pr-H), 7.25–7.38 (m, 5H, Ph-H and 8-H), 7.71 (d, *J* = 13.6 Hz, 1H, 5-H), 7.98 (s, 1H, 3-=CH)
4n	1.01–1.21 (m, 4H, *c*-Pr-H), 2.22 (s, 3H, CH_3), 3.06–3.24 (m, 8H, piperazine-H), 3.53–3.68 (m, 3H, 2-H and *c*-Pr-H), 7.05–7.46 (m, 3H, furyl-H, 8-H), 7.68 (d, *J* = 13.6 Hz, 1H, 5-H), 8.38 (d, *J* = 6.5 Hz, 1H, furyl-H), 7.93 (s, 1H, 3-=CH)
4o	1.03–1.23 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.12–3.28 (m, 8H, piperazine-H), 3.55–3.76 (m, 3H, 2-H and *c*-Pr-H), 7.25–8.12 (m, 5H, 8-H, 5-H, 3-=CH and pyridyl-H,), 8.24–9.06 (m, 2H, pyridyl-H)
4p	1.01–1.23 (m, 4H, *c*-Pr-H), 2.23 (s, 3H, CH_3), 3.15–3.27 (m, 8H, piperazine-H), 3.56–3.78 (m, 3H, 2-H and *c*-Pr-H), 7.25 (d, *J* = 7.6 Hz, 1H, 8-H), 7.87–8.93 (m, 6H, 5-H, 3-=CH and pyridyl-H)

仪 (德国 Bruker 公司); 2400-II元素分析仪 (美国 PE 公司)。所用环丙沙星 **1** 为商品, *N*-甲基环丙沙星按文献[20]的方法制备, 其他试剂均为分析纯。

1 化学合成

1.1 1-环丙基-6-氟-7-(4-甲基-哌嗪-基)-2,3-二氢-喹啉-4(1*H*)-酮 (3) 的合成 *N*-甲基环丙沙星 **2** (20.0 g, 58.0 mmol) 悬浮于无水甲醇 (1 000 mL) 中, 水浴常温搅拌下分批慢慢加入新购买的硼氢化钠粉末 (3.8 g, 100.0 mmol) (特别谨防冲料发生!)。待物料溶解后水浴加热至回流, 搅拌反应 2 h。减压蒸除溶剂, 加入饱和食盐水 (500 mL), 充分振荡至黏稠物完全分散, 转入烧杯中放置固化。过滤, 用水洗至中性, 干燥。粗品悬浮于蒸馏水 (200 mL) 中, 慢慢滴加浓盐酸至粗品溶解, 加入活性炭 2.0 g, 搅拌脱色 1 h。滤液用浓氨水碱化至 pH 9.0, 放置析出固体。过滤, 用水洗至中性, 自然干燥。用正己烷重结晶, 干燥, 得淡黄色针状结晶物 **2**。

1.2 1-环丙基-6-氟-7-(4-甲基-哌嗪-1-基)-3-芳苄叉基-2,3-二氢喹啉-4(1*H*)-酮 (4a～4p) 的合成通法 中间体 **3** (2.0 g, 6.6 mmol) 和新的芳香醛 (10.0 mmol) 依次溶于无水乙醇 (30 mL) 与氢氧化钠 (1.0 g, 13.0 mmol) 的醇钠溶液中, 常温磁力搅拌反应至原料 **3** 消失。减压蒸除溶剂, 剩余物加入饱和食盐水 (50 mL), 用浓盐酸调 pH 2.0, 用乙酸乙酯 (3×30 mL) 提取除去过量的醛。水相用浓氨水碱化至 pH 10.0, 放置析出固体。过滤, 用水洗至中性, 干燥。粗品用乙酸乙酯–无水乙醇混合溶剂重结晶, 得淡黄色针状结晶目标化合物 **4a**～**4p**。

2 体外抗癌细胞增殖活性

对合成的 16 个新目标化合物 **4a**～**4p** 及对照环丙沙星 (ciprofloxacin, **1**) 和结构类似抗癌药物多柔比星

Table 3 The *in vitro* antitumor activity of the target compounds **4a**–**4p** against the tested cancer cells ($n = 3$, $\bar{x} \pm s$)

Compd.	Ar	IC_{50}/μmol·L^{-1}		
		Hep-3B	Capan-1	HL60
4a	C_6H_5	15.8 ± 1.4	10.4 ± 0.7	27.5 ± 1.2
4b	4-CH_3O-C_6H_4	25.6 ± 1.7	15.8 ± 1.1	28.6 ± 1.5
4c	2-CH_3O-C_6H_4	16.5 ± 1.2	12.4 ± 1.2	25.3 ± 1.2
4d	4-CH_3-C_6H_4	23.7 ± 1.1	16.4 ± 1.5	28.6 ± 2.0
4e	3-CH_3-C_6H_4	20.5 ± 1.0	13.8 ± 0.7	26.3 ± 1.0
4f	3,4-OCH_2O-C_6H_3	25.6 ± 1.3	17.6 ± 0.6	31.6 ± 1.5
4g	3,4,5-$(CH_3O)_3$-C_6H_2	30.6 ± 1.8	21.5 ± 1.7	38.6 ± 2.0
4h	4-F-C_6H_4	8.7 ± 0.6	5.8 ± 0.6	15.7 ± 1.3
4i	3-F-C_6H_4	7.6 ± 1.0	6.0 ± 0.7	14.6 ± 1.4
4j	4-Cl-C_6H_4	5.6 ± 0.5	2.7 ± 0.6	10.6 ± 1.0
4k	3-Cl-C_6H_4	6.8 ± 0.7	3.6 ± 0.3	13.6 ± 1.1
4l	4-Br-C_6H_4	6.8 ± 0.8	7.2 ± 0.6	13.7 ± 1.5
4m	3-Br-C_6H_4	7.2 ± 0.5	8.5 ± 0.7	15.0 ± 1.2
4n	2-Furyl	6.7 ± 0.5	8.2 ± 0.8	13.8 ± 0.5
4o	3-Pyridyl	5.8 ± 0.6	7.6 ± 0.8	12.3 ± 0.6
4p	4-Pyridyl	7.0 ± 0.6	11.5 ± 0.6	15.6 ± 1.0
DOX		3.0 ± 0.6	2.2 ± 0.4	3.5 ± 0.6
1		>100	>100	>100

(doxorubicin, DOX) 用 DMSO 配成 1.0×10^{-2} mol·L^{-1} 浓度的储备液, 用 RPMI-1640 稀释到所需浓度 (50、10、5、1.0、0.1×10^{-6} mol·L^{-1})。取对数生长期的人肝癌 Hep-3B 细胞、人胰腺癌 Capan-1 细胞及人白血病 HL60 细胞分别以每孔 5 000 个细胞接种于 96 孔板, 培养隔夜后, 加入不同浓度的上述供试化合物溶液, 继续培养 48 h 后弃去培养基。每孔加入 1 g·L^{-1} MTT 溶液 100 μL, 继续培养 4 h 后弃上清液。每孔加入二甲基亚砜 150 μL, 轻轻振荡 30 min, 用酶标仪在 570 nm 波长处测其吸光度值。计算各组对癌细胞的抑制率: 抑制率% = [(1−实验组吸光度值)/对照组吸光度值]×100%。然后以各药物浓度的对数值对各浓度下的抑制率作线性回归, 得浓度-效应方程, 以此计算出各供试化合物对实验癌细胞的半数抑制浓度 (IC_{50})。所有实验在相同条件下重复进行 3 次, 最终结果以 Mean ± SD 表示。

作者贡献: 张会丽负责化合物的合成与结构表征; 姜亚玲负责化合物的合成; 赵辉负责药理实验与论文写作; 黄文龙负责课题设计; 胡国强为论文责任负责人。

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