氟喹诺酮 C-3 羧基等排体的合成及抗肿瘤活性:均三唑-噁二唑甲硫醚曼尼希碱衍生物

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摘 要 为寻找抗肿瘤氟喹诺酮 C-3 羧酸等排体的有效优化策略,基于 C-3 均三唑-噁二唑甲硫醚(**6a**~**6j**)结构特征,在培氟沙星(**1**)羧基等排体均三唑环上发生氨甲基化反应得新的曼尼希碱目标化合物(**7a**~**7j**),其结构经元素分析和光谱数据确证。用 MTT 方法评价了硫醚及其曼尼希碱化合物体外对 SMMC-7721、L1210 和 HL60 3 种肿瘤细胞的生长抑制活性。结果表明,硫醚及其曼尼希碱对 3 种肿瘤细胞的生长抑制活性不但显著强于母体化合物 **1**,而且曼尼希碱的活性也高于其相应硫醚的活性,尤其对肝癌 SMMC-7721 细胞的活性明显高于对白血病细胞 L1210 和 HL60 的活性,显示出了一定的抗肿瘤选择性。

关键词 氟喹诺酮;均三唑;噁二唑;生物电子等排体;合成;硫醚;曼尼希碱;抗肿瘤活性

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Synthesis and antitumor activities of fluoroquinolone C-3 isosteres(IV): *s*-triazole-oxadiazole methylsulfide Mannich-base derivatives

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Abstract To search for an efficient modification strategy for a bioisostere of the C-3 carboxylic acid group of antitumor fluoroquinolones, an aminomethylation reaction based on the structural characteristics of the C-3 *s*-triazole-oxadiazole sulfides(**6a-6j**) was carried out on a five-member azole ring of *s*-triazole to give 1-ethyl-6-fluoro-7-(4-methyl-piperazin-1-yl) -3-[1-dimethy-l amino-methyl-5-(5-substituted-phenyl-[1,3,4] oxadia-zol-2-yl methylsulfanyl) -1*H*-[1, 2, 4] -triazol-3-yl] -quinolin-4 (1*H*) -ones (**7a-7j**) as novel C-3 *s*-triazole-oxadiazole sulfide Mannich-base derivatives starting from pefloxacin(**1**). The structures of the title compounds were characterized by elemental analysis and spectral data and their *in vitro* antitumor activity against SMMC-7721, L1210 and HL60 cell lines was evaluated by a MTT assay. The results showed that the of sulfides(**6a-6j**) and their corresponding Mannich-base compounds(**7a-7j**) had more potent inhibitory activity than the compound **1**, and the Mannich-base compound **7** also exhibited more potent cytotoxicity than the corresponding compound **6**, especially both had better activity against SMMC-7721 cell line than the other cancer cell lines.

Key words fluoroquinolone; *s*-triazole; oxadiazole; synthesis; sulfide; bioisostere; Mannich-base; antitumor activity

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新药创新源于苗头化合物的发现,而后续对其优化是促成其向先导化合物转化的重要途径[1],而基于机制或结构的药物设计是发现苗头化合物的有效方法[2]。前期研究工作表明,拓扑异构酶

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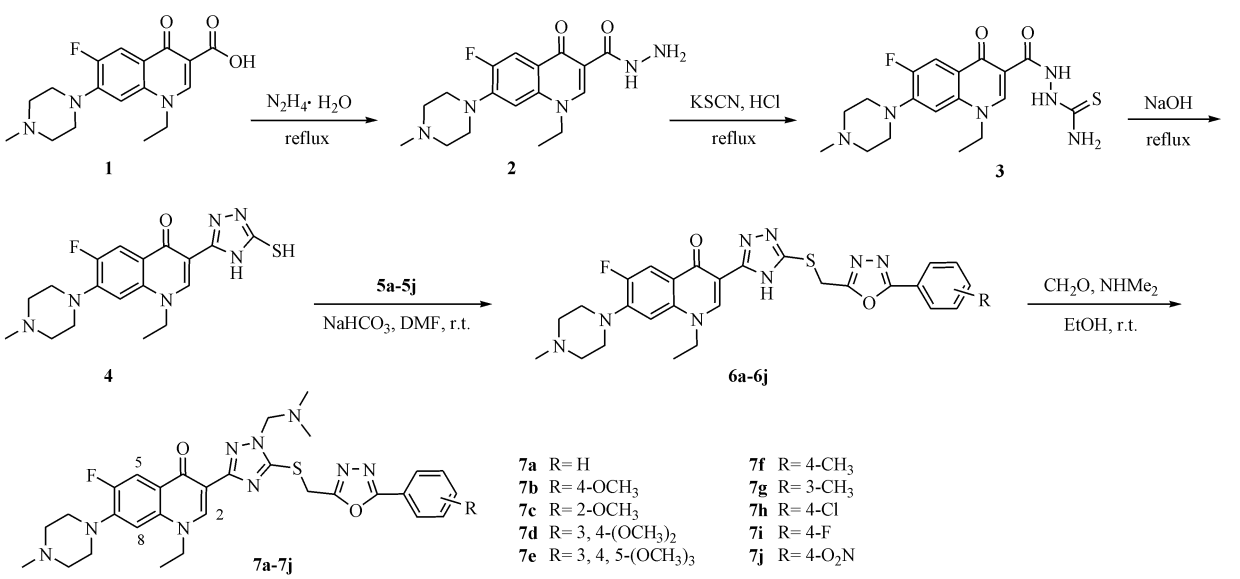
不但是抗肿瘤药物的重要作用靶[3],也是抗生素氟喹诺酮羧酸的作用靶酶[4],而且二者在功能和序列上具有相似性[4]。因此,氟喹诺酮羧酸通过结构修饰可转化为抗肿瘤药氟喹诺酮[5]。事实上,已有的研究表明,氟喹诺酮C-3位羧基并非是抗肿瘤活性所必需的药效团,可被其生物电子等排体如酰腙[6]、唑杂环[7]或唑稠杂环[8]替代。但对选择的等排体如何优化,进一步提高化合物的抗肿瘤活性、促进其向成药性方向发展仍是目前亟待解决的问题。为此,在前期研究的五元唑杂环均三唑硫醇作为氟喹诺酮羧酸培氟沙星(**1**)C-3羧基等排体衍生的C-3均三唑-噁二唑硫醚衍生物(**6a~6j**)具有潜在的抗肿瘤活性研究基础上[9],通过对均三唑环的结构特征分析发现,唑环中的N-H可作为活泼的H供体参与氨甲基化反应,可方便地在均三唑环上引入重要的药效团曼尼希碱[10],从而实现对C-3等排体的进一步优化到C-3均三唑-噁二唑硫醚曼尼希碱衍生物(**7a~7j**),并通过对新目标化合物**7a~7j**活性评价,为进一步优化C-3等排体提供有效途径和方法。

1 合成路线

目标化合物**7a~7j**的制备见路线1。培氟沙星经胼解、与硫氰化钾缩合、环合等步骤得到关键中间体C-3均三唑硫醇**4**,它分别与2-芳基-[1,3,4]-噁二唑-5-氯甲烷**5a~5j**发生亲核取代反应到C-3均三唑-噁二唑硫醚产物**6a~6j**[9],然后均三唑环N-H与甲醛和二甲胺进行氨甲基化反应得到C-3均三唑-噁二唑硫醚曼尼希碱目标化合物**7a~7j**。



Scheme 1 Synthetic route for Mannich-base derivatives **7a-7j** from sulfides **6a-6j**

2 实验部分

2.1 化学合成

上海申光WK-1B数字熔点仪;ESI-MS由德国Bruker公司Esquire LC型质谱仪测定;^{1}H NMR为Bruker AM-400型核磁共振仪测定,DMSO-d_6为溶剂,TMS为内标。美国Nicolet AVATAR360红外光谱仪,KBr压片;美国PE PE2400-Ⅱ元素分析仪。培氟沙星**1**为市售商品,活性N-H均三唑-噁二唑硫醚产物**6a~6j**按前期研究[9]方法制备,其余试剂为分析纯。

2.2 1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-(5-取代苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基]-喹啉-4(1*H*)-酮(**7a~7j**)的合成通法

化合物**6** 1.0 g悬浮于乙醇25 mL中,加入二甲胺盐酸盐0.2 g(2.4 mmol),回流1 h后滴加40%甲醛溶液(1.0 mL),搅拌至澄清。放置冰箱析出固体,过滤,乙醇洗涤。固体溶解在去离子水中(20 mL),氨水碱化至pH 8.0,氯仿提取,无水硫酸钠干燥,减压蒸干溶剂,无水乙醇重结晶,得类白色固体硫醚曼尼希碱**7a~7j**。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-(5-苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基]-喹啉-4(1*H*)-酮(**7a**),收率:54%,mp:157~159 ℃;

^{1}H NMR(DMSO-d_6) δ:8.63(1H,s,2-H),7.84 ~ 7.42(7H,m,5-,8-,Ph-H),5.14(2H,s,NCH_2),4.70(2H,s,SCH_2),4.46(2H,q,J = 7.2 Hz,NCH_2),3.46 ~ 3.15(8H,m,2 × NCH_2CH_2),2.35 ~ 2.23(9H,br,3 × N-CH_3),1.38(3H,t,J = 7.2 Hz,CH_3);EI-MS(m/z):604[M + H]$^+$;Anal. calcd. for $C_{30}H_{34}FN_9O_2S$(%):C 59.69,H 5.68,N 20.88;Found:C 59.92,H 5.47,N 21.04。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-(5-对甲氧苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基]-喹啉-4(1*H*)-酮(**7b**),收率:63%,mp:163 ~ 165 ℃;^{1}H NMR(DMSO-d_6) δ:8.72(1H,s,2-H),7.91 ~ 7.55(6H,m,5-,8-,Ph-H),5.17(2H,s,NCH_2),4.80(2H,s,SCH_2),4.47(2H,q,J = 7.1 Hz,NCH_2),3.86(3H,s,OCH_3),3.53 ~ 3.27(8H,m,2 × NCH_2CH_2),2.34 ~ 2.25(9H,br,3 × N-CH_3),1.45(3H,t,J = 7.1 Hz,CH_3);EI-MS(m/z):634[M + H];Anal. calcd. for $C_{31}H_{36}FN_9O_3S$(%):C 58.75,H 5.73,N 19.89;Found:C 58.96,H 5.50,N 19.72。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-(5-邻甲氧苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基]-喹啉-4(1*H*)-酮(**7c**),收率:51%,mp:135 ~ 137 ℃;^{1}H NMR(DMSO-d_6) δ:8.74(1H,s,2-H),8.13 ~ 7.62(6H,m,5-,8-,Ph-H),5.21(2H,s,NCH_2),4.84(2H,s,SCH_2),4.48(2H,q,J = 7.1 Hz,NCH_2),3.87(3H,s,OCH_3),3.55 ~ 3.23(8H,m,2 × NCH_2CH_2),2.35 ~ 2.27(9H,br,3 × N-CH_3),1.46(3H,t,J = 7.1 Hz,CH_3);EI-MS(m/z):634[M + H]$^+$;Anal. calcd. for $C_{31}H_{36}FN_9O_3S$(%):C 58.75,H 5.73,N 19.89;Found:C 58.95,H 5.45,N 20.12。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-{5-(3,4-二甲氧苯基)}-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7d**),收率:67%,mp:142 ~ 144 ℃;^{1}H NMR(DMSO-d_6) δ:8.76(1H,s,2-H),8.15 ~ 7.58(5H,m,5-,8-,Ph-H),5.23(2H,s,NCH_2),4.86(2H,s,SCH_2),4.47(2H,q,J = 7.1 Hz,NCH_2),3.89 and 3.86(6H,2s,2 × OCH_3),3.53 ~ 3.18(8H,m,2 × NCH_2CH_2),2.37 ~ 2.28(9H,br,3 × N-CH_3),1.45(3H,t,J = 7.1 Hz,CH_3);EI-MS(m/z):664[M + H]$^+$;Anal. calcd. for $C_{32}H_{38}FN_9O_4S$(%):C 58.75,H 5.77,N 18.99;Found:C 58.96,H 5.58,N 19.25。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-{5-(3,4,5-三甲氧苯基)}-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7e**),收率:64%,mp:146 ~ 148 ℃;^{1}H NMR(DMSO-d_6) δ:8.68(1H,s,2-H),8.13 ~ 7.57(4H,m,5-,8-,Ph-H),5.24(2H,s,NCH_2),4.88(2H,s,SCH_2),4.48(2H,q,J = 7.1 Hz,NCH_2),3.86 and 3.91(9H,2s,3 × OCH_3),3.56 ~ 3.17(8H,m,2 × NCH_2CH_2),2.36 ~ 2.27(9H,br,3 × N-CH_3),1.46(3H,t,J = 7.1 Hz,CH_3);EI-MS(m/z):694[M + H]$^+$;Anal. calcd. for $C_{33}H_{40}FN_9O_5S$(%):C 57.13,H 5.81,N 18.17;Found:C 57.39,H 5.59,N 18.42。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-[5-对甲苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7f**),收率:61%,mp:158 ~ 160 ℃;^{1}H NMR(DMSO-d_6) 8.61(1H,s,2-H),7.78 ~ 7.35(6H,m,5-,8-,Ph-H),5.18(2H,s,NCH_2),4.72(2H,s,SCH_2),4.45(2H,q,J = 7.2 Hz,NCH_2),3.38 ~ 3.06(8H,m,2 × NCH_2CH_2),2.34 ~ 2.22(12H,m,Ph-CH_3,3 × N-CH_3),1.37(3H,t,J = 7.2 Hz,CH_3);EI-MS(m/z):618[M + H]$^+$;Anal. calcd. for $C_{31}H_{36}FN_9O_2S$(%):C 60.27,H 5.87,N 20.41;C 60.48,H 5.64,N 20.63。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-[5-邻甲苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7g**),收率:53%,mp:145 ~ 147 ℃;^{1}H NMR(DMSO-d_6) 8.63(1H,s,2-H),7.76 ~ 7.46(6H,m,5-,8-,Ph-H),5.20(2H,s,NCH_2),4.73(2H,s,SCH_2),4.47(2H,q,J = 7.2 Hz,2H,NCH_2),3.46 ~ 3.13(8H,m,2 × NCH_2CH_2),2.36 ~ 2.27(12H,m,12H,Ph-CH_3 and 3 × N-CH_3),1.42(3H,t,J = 7.2 Hz,CH_3);EI-MS(m/z):618[M + H]$^+$;Anal. calcd. for $C_{31}H_{36}FN_9O_2S$(%):C 60.27,H 5.87,N 20.41;Found:C 60.52,H 5.94,N 20.58。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-[5-邻甲苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7h**),收率:57%,mp:153 ~ 155 ℃;^{1}H NMR(DMSO-d_6) δ:8.73(1H,s,2-H),7.93 ~ 7.47(6H,m,5-,8-,Ph-H),5.23(2H,s,NCH_2),4.78(2H,s,SCH_2),4.51(2H,q,J = 7.1 Hz,NCH_2),3.53 ~ 3.25(8H,m,2 × NCH_2CH_2),3.37 ~ 2.24(9H,br,3 × N-CH_3),1.47(3H,t,J = 7.1 Hz,CH_3);EI-MS(m/z):638,^{35}Cl[M + H]$^+$;Anal. calcd. for $C_{30}H_{33}ClFN_9O_2S$(%):C 56.46,H 5.21,N 19.75;Found:C 56.68,H 5.07,N 19.88。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-[5-对氟甲苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7i**),收率:68%,mp:182 ~ 184 ℃;^{1}H NMR(DMSO-d_6) δ:8.76(1H,s,1H,2-H),8.03 ~ 7.52(6H,m,5-,8-,Ph-H),5.26(2H,s,NCH_2),4.81(2H,s,SCH_2),4.50(2H,q,J = 7.2 Hz,2H,NCH_2),3.57 ~ 3.34(8H,m,2 × NCH_2CH_2),3.36 ~ 2.28(9H,br,3 × N-CH_3),1.52(3H,t,7.2 Hz,CH_3);EI-MS(m/z):622[M + H]$^+$;Anal. calcd. for $C_{30}H_{33}F_2N_9O_2S$(%):C 57.96,H 5.35,N 20.28;Found:C 58.13,H 5.17,N 20.50。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[1-二甲氨基甲基-5-[5-对硝基甲苯基-1,3,4-噁二唑-2-甲硫基)-1*H*-1,2,4-三唑-3-基}-喹啉-4(1*H*)-酮(**7j**),收率:63%,mp:189 ~ 191 ℃;^{1}H NMR(DMSO-d_6) δ:8.84(1H,s,1H,2-H),8.08 ~ 7.56(6H,m,5-,8-,Ph-H),5.28(2H,s,NCH_2),

4.83(2H,s,SCH_2),4.55(2H,q,J=7.1 Hz,NCH_2),3.54~3.36(8H,m,2×NCH_2CH_2),3.38~2.34(9H,br,3×N-CH_3),1.54(3H,t,7.1 Hz,CH_3);EI-MS(m/z):649[M+H]$^+$;Anal. calcd. for $C_{30}H_{33}FN_{10}O_4S$(%):C 55.55,H 5.13,N 21.59;Found:C 55.72,H 5.28,N 21.82。

2.3 抗肿瘤活性评价

对合成的10个双杂环硫醚**6a~6j**及其曼尼希碱目标化合物**7a~7j**和对照蒽醌类抗肿瘤药阿霉素(DOX)及先导物培氟沙星(PFX)用DMSO配成1.0×10^{-2} mol/L浓度的储备液,用RPMI-1640培养液稀释到所需浓度。取对数生长期的人肝癌细胞(SMMC-7721)以每孔5千个细胞的量接种于96孔板。培养隔夜后,加入不同浓度的上述化合物。48 h后弃去培养基,每孔加入1 g/L MTT溶液100 μL,继续培养4 h后弃上清液,每孔加入DMSO 150 μL,轻轻振荡30 min,用酶标仪在570 nm波长处测其吸收度。取对数生长期的鼠白血病细胞(L1210)和人白血病细胞(HL60),以每孔7千个细胞的量接种于96孔板,随后加入不同浓度的上述化合物,48 h后每孔加入5 g/L MTT溶液10 μL,继续培养4 h后加入10% SDS溶液100 μL培养过夜,用酶标仪在570 nm波长处测其吸收度,计算细胞抑制率和半数机制浓度IC_{50},结果见表2。

Table 2 Inhibitory activities of sulfides(**6a-6j**) and Mannich-base compounds(**7a-7j**) against SMMC-7721,L1210 and HL60 tumor cells

Compd.	IC_{50}/(μmol/L)		
	SMMC-7721	L1210	HL60
6a/7a	10.6/6.4	12.7/8.4	15.3/10.6
6b/7b	11.4/4.7	14.6/7.6	17.4/11.8
6c/7c	3.8/1.2	5.6/4.2	7.6/5.3
6d/7d	12.6/7.8	15.7/10.3	17.2/12.0
6e/7e	13.6/8.7	20.6/12.6	24.5/14.7
6f/7f	12.5/10.3	26.3/15.8	32.4/16.3
6g/7g	12.3/7.8	18.5/11.3	23.4/12.5
6h/7h	14.5/8.2	20.7/15.7	25.7/17.4
6i/7i	2.7/1.5	4.7/3.8	6.2/5.2
6j/7j	15.8/11.6	30.5/20.4	35.2/24.5
Doxorubicin	2.3	1.2	2.6
Pefloxacin	>150	>150	>150

体外抗肿瘤筛选结果表明,化合物**7a~7j**对3种试验肿瘤细胞株的半数抑制浓度均高于相应前体硫醚**6a~6j**,尤其对肝癌细胞(SMMC-7721)的抑制活性高于对白血病细胞(L1210和HL60)的活性,显示出了一定的选择性。初步的构效关系表明,无论是硫醚或硫醚曼尼希碱,其IC_{50}均低于25.0 μmol/L,虽活性低于对照阿霉素,但显著强于先导物培氟沙星的活性(>150 μmol/L),表明羧基并非是抗肿瘤必要的,可被均三唑杂环等排体替代;同时,比较硫醚**6**和硫醚曼尼希碱**7**的活性可知,硫醚**6**的C-3杂环被功能基曼尼希碱修饰得到的化合物**7**的活性有显著性提高,表明在C-3杂环上用功能化的基团修饰有利于提高抗肿瘤活性,而随着修饰杂环噁二唑的取代基体积的增大,活性降低,这为由抗生素氟喹诺酮向抗肿瘤活性药的转化提供了重要的修饰途径和方法。

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