·论 文·

C-3 噻唑并均三唑取代的培氟沙星衍生物的合成及抗肿瘤活性(Ⅸ)

闫 强[1],吴书敏[1],倪礼礼[1],谢玉锁[1],高留州[1],黄文龙[2],刘英杰[3*],胡国强[1**]

([1]河南大学化学生物学研究所,开封 475001;[2]中国药科大学新药研究中心,南京 210009;
[3]河南大学医学院微生物教研室,开封 475001)

摘 要 为寻找抗肿瘤氟喹诺酮类化合物的新方法,用稠杂环核作为培氟沙星(**1**)C-3 羧基的生物电子等排体,设计合成了 12 个新的噻唑并[3,2-b][1,2,4]三唑类目标化合物(**6a ~ 6l**),其结构经元素分析和光谱数据确证。选择 SMMC-7721、L1210 和 HL60 3 种肿瘤细胞株进行体外抗增殖活性实验,结果表明目标化合物的抗肿瘤活性高于先导化合物 **1** 和相应的开环中间体硫醚酮(**5a ~ 5l**),其中苯环上有羟基或氟原子取代的目标化合物对 SMMC-7721 肿瘤细胞显示出较强的活性。基于此,噻唑并均三唑稠杂环可作为氟喹诺酮 C-3 羧基的等排体用于抗肿瘤氟喹诺酮类化合物的设计。

关键词 氟喹诺酮;均三唑;噻唑;噻唑并三唑;抗肿瘤活性

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Synthesis and antitumor activity of C-3 thiazolo [3, 2-b] [1, 2, 4] triazole-substituted pefloxacin derivatives

YAN Qiang[1], WU Shumin[1], NI Lili[1], XIE Yusuo[1], GAO Liuzhou[1], HUANG Wenlong[2], LIU Yingjie[3*], HU Guoqiang[1**]

[1] Institute of Chemical Biology, Henan University, Kaifeng 475001; [2] Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009; [3] Department of Microbiology, School of Medicine, Henan University, Kaifeng 475001, China

Abstract To search for fluoroquinolones (FQs) with antitumor activity, the C-3 carboxylic acid group of pefloxacin (**1**) was replaced by fused heterocyclic core, and twelve novel thiazolo[3,2-b][1,2,4]triazole heterocycles (**6a-6l**) were designed and synthesized. The structures of target compounds were characterized by elemental analysis and spectral data. The results of the *in vitro* antiproliferative effect on SMMC-7721, L1210 and HL60 cell lines showed that the title compounds exhibited more significant antitumor activity than both of the pefloxacin and the corresponding opening-ring intermediates (**5a-5l**). Among them, the target compounds which possess a benzene ring bearing a hydroxyl group (**6e**) or a fluorine atom (**6j**) exhibited more potent antiproliferative effect on SMMC-7721 cells than other compounds. Therefore, the antitumor fluoroquinolones can be designed by replacing the C-3 carboxylic acid group of fluoroquinolones with the thiazolo [3,2-b][1,2,4] triazole moiety.

Key words fluoroquinolone; *s*-triazole; thiazole; thiazolotriazole; antitumor activity

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新药创新起源于先导化合物的发现,而基于现有药物的结构进行药效团的拼合和优势骨架的迁越是构建药物化学分子结构的有效手段[1]。1-取代-6-氟-喹啉-4-酮-3-羧酸是抗菌氟喹诺酮药物分

收稿日期 2015-01-14 * **通信作者** * Tel:0371 -23880585 E-mail:L-yingjie@henu.edu.cn
** Tel:0371 -23880680 E-mail:hgqxy@sina.com

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子的优势骨架[2],五元唑杂环及其稠杂类因具有广泛的药理活性在新药创制中常被用作有效的药效团来构建结构多样的候选化合物[3]。其中,前文已发现均三唑杂环用作氟喹诺酮 C-3 羧酸基的等排体,并对其侧链进行功能化修饰可产生抗肿瘤活性的 C-3 杂环衍生物[4-5]。然而,在众多的唑稠杂环[6]中,虽然均三唑与噻唑相稠合的噻唑并三唑衍生物因有抗菌、抗肿瘤、抗炎等多种生物活性而在新药研发中备受关注[7],但该稠杂环核作为 C-3 羧基等排体将会对抗肿瘤活性生产如何的影响,目前尚未见报道。基于此,为发现 C-3 羧基新的等排体,用噻唑并均三唑稠核替代 C-3 羧基,进而设计合成了氟喹诺酮 C-3 稠杂环类目标化合物(**6a ~ 6l**),并通过抗肿瘤活性的评价,初步判断设计方案的合理性。

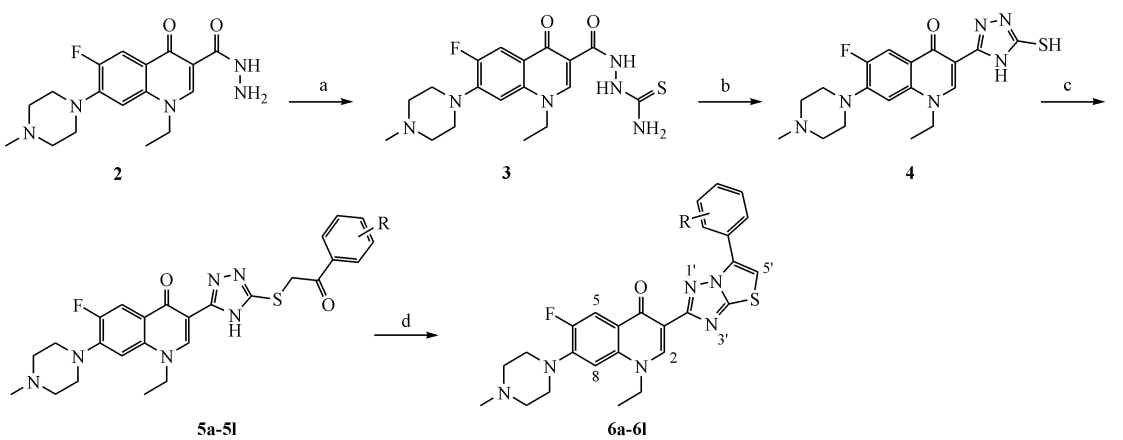
1　合成路线

目标化合物 **6a ~ 6l** 的合成见路线 1。培氟沙星酰肼 **2** 与硫氰化钾在酸性水溶液中发生缩合反应生成 C-3 酰胺基硫脲 **3**,接着化合物 **3** 在碱性水溶液中发生分子内环合反应得到 C-3 均三唑硫醇 **4**,它与溴代苯乙酮类发生亲核取代反应到 C-3 均三唑硫醚酮类中间体 **5a ~ 5l**。硫醚酮 **5a ~ 5l** 在多聚磷酸中发生分子内环合反应,得到相应的 C-3 噻唑并[3,2-b][1,2,4]三唑目标化合物 **6a ~ 6l**。

2　实验部分

2.1　材　料

WK-1B 数字熔点仪(上海申光仪器仪表有限公司);Esquire LC 型质谱仪、AM-400 型核磁共振仪(德国 Bruker 公司),TMS 为内标;2400-Ⅱ元素分析仪(美国 Perkin-Elmer 公司);680 型酶标仪(美国 Bio-Rad 公司)。C-3 均三唑硫醚酮 **5a ~ 5l** 按文献[8]的方法制备,其他试剂均为市售分析纯。



Scheme 1　Synthetic route of the title compounds(**6a-6l**) from acylhydrazine (**2**) of pefloxacin (**1**)

R:H(**a**);4-CH_3O(**b**);2-CH_3O(**c**);4-HO(**d**);2-HO(**e**);3,4-(OCH_2O)(**f**);3,4-(CH_3O)$_2$(**g**);4-OH-3-CH_3O(**h**);4-CH_3(**i**);4-F(**j**);4-Cl(**k**);4-O_2N(**l**)

a:KSCN,HCl-H_2O,reflux;b:NaOH,H_2O,reflux;c:Ar-$COCH_2Br$,EtOH,reflux;d:PPA,toluene,reflux

2.2　化学合成

1-乙基-6-氟-(4-甲基-哌嗪-基)-3-(6-苯基-噻唑并[3,2-b][1,2,4]三唑-2-基)-喹啉-4(1*H*)-酮(**6a**)的合成

1-乙基-6-氟-(4-甲基-哌嗪-基)-3-(5-苯甲酰甲硫基-4*H*-[1,2,4]三唑-3-基)-喹啉-4(1*H*)-酮(**5a**)(2.0 g,4.0 mmol)悬浮于多聚磷酸(5.0 g)和甲苯(20 mL)的混合液中,搅拌回流反应 24 h。反应液慢慢倾入冰水(50 mL)中,用碳酸氢钠慢慢调 pH 10.0,静置,分出有机层。水相用氯仿提取(310 mL),合并有机相,无水硫酸钠干燥。过滤,减压蒸除溶剂,用无水乙醇重结晶,得淡黄色结晶目标物 **6a**。由化合物 **5b ~ 5l** 按同法制备 1-乙基-6-氟-(4-甲基-哌嗪-基)-3-(6-取代苯基-噻唑并[3,2-b][1,2,4]三唑-2-基)-喹啉-4(1*H*)-酮(**6b ~ 6l**)目标物,其理化性质和 MS 数据见表 1、^{1}H NMR 数据见表 2。

Table 1 Physical constants and spectral data of the title compounds **6a-6l**

Compd.	Yield/%	mp/°C	Elemental analysis(%, Calcd.)			MS(*m/z*)
			C	H	N	[M+H]$^+$(Calcd.)
6a	91.0	186-188	64.13(63.92)	5.33(5.16)	17.38(17.20)	489(488.59)
6b	93.5	250-252	62.76(62.53)	5.04(5.25)	16.42(16.20)	519(518.62)
6c	86.4	236-238	62.80(62.53)	5.36(5.25)	16.37(16.20)	519(518.62)
6d	76.8	255-257	61.76(61.89)	4.84(4.99)	16.92(16.66)	505(504.59)
6e	68.7	226-228	62.06(61.89)	4.86(4.99)	16.87(16.66)	505(504.59)
6f	94.6	>260	60.71(60.89)	4.64(4.73)	16.03(15.78)	533(532.60)
6g	81.5	237-239	61.52(61.30)	5.12(5.33)	15.56(15.32)	549(548.64)
6h	80.6	>260	60.87(60.66)	4.87(5.09)	15.96(15.72)	535(534.62)
6i	86.0	211-214	64.73(64.52)	5.20(5.41)	16.95(16.72)	503(502.62)
6j	95.3	245-247	61.83(61.65)	4.97(4.78)	16.78(16.59)	507(506.58)
6k	86.8	225-227	59.88(59.71)	4.42(4.63)	16.38(16.07)	523(523.04)
6l	90.6	>260	58.76(58.53)	4.72(4.53)	18.64(18.37)	534(533.59)

Table 2 ^{1}H NMR data of the target compounds **6a-6l**

Compd.	^{1}H NMR(400 MHz, DMSO-d_6)
6a	1.41(3H,t,J=7.0 Hz,CH_3),2.46(3H,s,N-CH_3),3.25-3.82(8H,m,piperazine-H),4.50(2H,q,J=7.0 Hz,CH_2),7.17(1H,d,J=7.1 Hz,8-H),7.51-7.62(3H,m,Ph-H),7.91(1H,d,J=12.4 Hz,5-H),7.94(1H,s,5′-H),8.32(2H,d,J=7.6 Hz,Ph-H),8.71(1H,s,2-H)
6b	1.41(3H,t,J=7.0 Hz,CH_3),2.41(3H,s,N-CH_3),2.68-3.35(8H,m,piperazine-H),3.85(3H,s,OCH_3),4.50(2H,q,J=7.0 Hz,CH_2),7.08(1H,d,J=7.1 Hz,8-H),7.15(2H,d,J=8.8 Hz,Ph-H),7.76(1H,s,5′-H),7.86(1H,d,J=13.2 Hz,5-H),8.27(2H,d,J=8.8 Hz,Ph-H),8.67(1H,s,2-H)
6c	1.44(3H,t,J=7.0 Hz,CH_3),2.43(3H,s,N-CH_3),2.86-3.37(8H,m,piperazine-H),3.86(3H,s,OCH_3),4.52(2H,q,J=7.0 Hz,CH_2),7.15(1H,d,J=7.1 Hz,8-H),7.25-7.68(2H,m,Ph-H),7.82(1H,s,5′-H),7.87(1H,d,J=13.2 Hz,5-H),8.16-8.28(2H,m,Ph-H),8.43(1H,s,2-H)
6d	1.45(3H,t,J=7.0 Hz,CH_3),2.42(3H,s,N-CH_3),2.72-3.38(8H,m,piperazine-H),4.51(2H,q,J=7.0 Hz,CH_2),7.12(1H,d,J=7.1 Hz,8-H),7.26(2H,d,J=8.8 Hz,Ph-H),7.78(1H,s,5′-H),7.87(1H,d,J=13.2 Hz,5-H),8.35(2H,d,J=8.8 Hz,Ph-H),8.44(1H,s,2-H),10.53(1H,s,OH)
6e	1.46(3H,t,J=7.0 Hz,CH_3),2.42(3H,s,N-CH_3),3.07-3.65(8H,m,piperazine-H),4.54(2H,q,J=7.0 Hz,CH_2),7.18(1H,d,J=7.1 Hz,8-H),7.33-7.67(2H,m,Ph-H),7.78(1H,s,5′-H),7.86(1H,d,J=13.2 Hz,5-H),8.21-8.30(2H,m,Ph-H),8.64(1H,s,2-H)
6f	1.42(3H,t,J=7.0 Hz,CH_3),2.44(3H,s,CH_3),3.15-3.67(8H,m,piperazine-H),4.51(2H,q,J=7.0 Hz,CH_2),6.26(2H,s,OCH_2O),7.20-7.68(3H,m,Ph-H and 8-H),7.86(1H,d,J=13.2 Hz,5-H),7.96(1H,s,5′-H),8.23(1H,s,Ph-H),8.46(1H,s,2-H)
6g	1.44(3H,t,J=7.0 Hz,CH_3),2.43(3H,s,CH_3),3.18-3.67(8H,m,piperazine-H),3.86,3.88(6H,2s,2OCH_3),4.48(2H,q,J=7.0 Hz,CH_2),7.32-7.42(3H,m,Ph-H and 8-H),7.76(1H,s,5′-H),7.88(1H,d,J=13.2 Hz,5-H),8.30(1H,s,Ph-H),8.43(1H,s,2-H)
6h	1.45(3H,t,J=7.0 Hz,CH_3),2.46(3H,s,CH_3),3.20-3.68(8H,m,piperazine-H),3.87(3H,s,OCH_3),4.53(2H,q,J=7.0 Hz,CH_2),7.31-7.58(3H,m,Ph-H and 8-H),7.82(1H,s,5′-H),7.93(1H,d,J=13.2 Hz,5-H),8.32(1H,s,Ph-H),8.42(1H,s,2-H),10.55(1H,s,OH)
6i	1.54(3H,t,J=7.0 Hz,CH_3),2.40,2.41(6H,2s,2CH_3),2.68-3.30(8H,m,piperazine-H),4.42(2H,q,J=7.0 Hz,CH_2),6.76(2H,d,J=8.0 Hz,Ph-H),7.32(1H,d,J=6.8 Hz,8-H),7.88-8.10(3H,m,Ph-H and 5′-H),8.19(1H,d,J=13.2 Hz,5-H),8.48(1H,s,2-H)
6j	1.57(3H,t,J=7.0 Hz,CH_3),2.42(3H,s,CH_3),2.78-3.36(8H,m,piperazine-H),4.47(2H,q,J=7.0 Hz,CH_2),7.28(2H,d,J=8.0 Hz,Ph-H),7.38(1H,d,J=6.8 Hz,8-H),7.87(1H,s,5′-H),8.16(1H,d,J=13.2 Hz,5-H),8.36(2H,d,J=8.0 Hz,Ph-H),8.47(1H,s,2-H)
6k	1.53(3H,t,J=7.0 Hz,CH_3),2.41(3H,s,CH_3),2.68-3.37(8H,m,piperazine-H),4.51(2H,q,J=7.0 Hz,CH_2),7.32(2H,d,J=8.0 Hz,Ph-H),7.53(1H,d,J=6.8 Hz,8-H),7.88(1H,s,5′-H),8.12(1H,d,J=13.2 Hz,5-H),8.33(2H,d,J=8.0 Hz,Ph-H),8.45(1H,s,2-H)
6l	1.42(3H,t,J=7.0 Hz,CH_3),2.30(3H,s,CH_3),2.60-3.27(8H,m,piperazine-H),4.46(2H,q,J=7.0 Hz,CH_2),7.46(2H,d,J=8.0 Hz,Ph-H),7.56(1H,d,J=6.8 Hz,8-H),7.86(1H,s,5′-H),8.07(2H,d,J=8.0 Hz,Ph-H),8.19(1H,d,J=13.2 Hz,5-H),8.47(1H,s,2-H)

2.3　抗肿瘤活性评价

对合成的 12 个新稠杂环 C-3 噻唑并均三唑目标化合物(**6a**~**6l**)及其相应的开环硫醚酮中间体(**5a**~**5l**)和对照蒽醌类抗肿瘤药阿霉素(DOX)及母体培氟沙星(**1**)用 DMSO 配成 1.0×10^{-2} mol/L 的储备液,按文献[9]的方法测定对人肝癌细胞(SMMC-7721)、鼠白血病细胞(L1210)和人白血病细胞(HL60)的半数抑制浓度(IC_{50}),结果见表 3。

Table 3　Antiproliferative activity of compounds **5a-5l** and **6a-6l** against SMMC-7721, L1210 and HL60 tumor cells

Compd.	IC_{50}/(μmol/L)		
	SMMC-7721	L1210	HL60
5a/6a	33.7/18.7	42.8/26.5	48.3/31.7
5b/6b	38.6/20.4	51.6/31.6	43.4/26.3
5c/6c	27.6/12.7	38.5/25.6	34.2/23.8
5d/6d	25.3/10.4	30.8/11.7	28.3/13.2
5e/6e	21.7/8.5	26.2/14.8	26.8/8.6
5f/6f	41.5/26.8	53.2/30.5	45.3/27.3
5g/6g	36.2/17.8	47.3/24.6	42.8/25.6
5h/6h	22.6/11.3	27.5/15.0	25.8/14.8
5i/6i	41.6/21.3	47.8/25.6	45.8/25.7
5j/6j	18.4/7.3	21.6/8.9	19.3/10.2
5k/6k	41.7/12.5	52.4/14.3	45.2/27.1
5l/6l	45.2/30.6	56.8/37.3	48.7/27.8
Doxorubicin	2.8	2.0	3.0
1	>100	>100	>100

体外抗增殖活性结果(表 1)表明,12 个 C-3 均三唑硫醚酮中间体(**5a**~**5l**)和 12 个 C-3 噻唑并均三唑稠杂环目标化合物(**6a**~**6l**)对 3 种试验肿瘤细胞株的 IC_{50}虽然高于对照阿霉素,但均低于其前体药培氟沙星的 IC_{50},表明均三唑杂环及其稠杂环均可作为 C-3 羧基的等排体。初步的构-效关系表明,稠杂环衍生物(**6a**~**6l**)的活性虽然强于相应开环均三唑侧链硫醚酮(**5a**~**5l**)的抗肿瘤活性,但弱于前文 C-3 均三唑硫醚酮缩氨基硫脲类的活性[9],可能缩氨基硫脲侧链部分更有利于与靶点结合,进而产生较强的活性。同时,在目标化合物中,苯环带有羟基或氟原子的化合物对 SMMC-7721 细胞显示出较强的活性。基于此,用稠杂环作为氟喹诺酮 C-3 羧基的等排体可能是转化其抗菌活性到其抗肿瘤活性的又一新策略。

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