·论 文·

氟喹诺酮 C-3 羧基等排体的合成及抗肿瘤活性Ⅲ. 均三唑噁二唑甲硫醚衍生物

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摘 要 为寻找由氟喹诺酮抗菌活性到抗肿瘤活性转化的有效途径,用均三唑和噁二唑分别作为培氟沙星 C-3 羧基的等排体和修饰基,设计合成了 10 个未见文献报道的双杂环硫醚 1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[5-(5-芳基-[1,3,4]噁二唑-2-甲硫基)-4*H*-[1,2,4]-三唑-3-基]-喹啉(1*H*)-4-酮新化合物(**7a**~**7j**)。结果显示:对 L1210、HL60 和 CHO 3 种肿瘤细胞的体外抑制活性显著高于母体培氟沙星,表明 C-3 羧基不是抗肿瘤活性所必需的药效团,可被杂环等排体替换,进一步扩展了结构修饰的途径。

关键词 氟喹诺酮;均三唑;噁二唑;硫醚;生物电子等排体;合成;抗肿瘤活性

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Synthesis and antitumor activity of fluoroquinolone C-3 isostere Ⅲ: *s*-triazole oxadiazole methylsulfide derivatives from pefloxacin

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Abstract To discover an efficient route for the conversion of antibacterial fluoroquinolone to antitumor activity, *s*-triazole ring as an isostere modified by an oxadiazole ring corresponding to the C-3 carboxylic acid group for pefloxacin resulted in ten new title compounds, 1-ethyl- 6-fluoro-(4-methylypiperazin-1-yl) -3-[5-(5-aryl-[1,3,4] oxadiazol-2-methylsulfanyl) -4*H*-[1,2,4] -triazol-3-yl] -quinolin(1*H*) -4-ones(**7a**-**7j**). Their structures were characterized by corresponding spectral data. The *in vitro* antitumor activity of the title compounds against L1210, HL60 and CHO cell lines exhibited significantly higher potency than parent pefloxacin. Thus, it suggests that it is necessary to retain a heterocycle instead of a C-3 carboxyl for antitumor fluoroquinolone compounds.

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

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基于机制的药物设计是发现先导化合物的有效途径,对其结构优化是促其向成药性发展的关键环节[1]。基于拓扑异构酶作为抗生素氟喹诺酮和许多抗肿瘤药物的共同作用靶酶[2],可转化氟喹诺酮抗菌活性到抗肿瘤活性,并已发现 C-3 羧基虽然是抗菌活性所必需的,但并非是抗肿瘤活性所必要的药效团,可被其生物电子等排体酰腙和杂环替代[3-4],但哪些杂环是最佳的等排体及对等排体如何进行结构优化,目前尚未见系统的研究。考虑到五元唑杂环类化合物具有多种生物活性,在新药设计中也是最常见的药效团之一[5-6],为此,选择均三唑杂环作为培氟沙星 C-3 羧基的等排体、噁二唑

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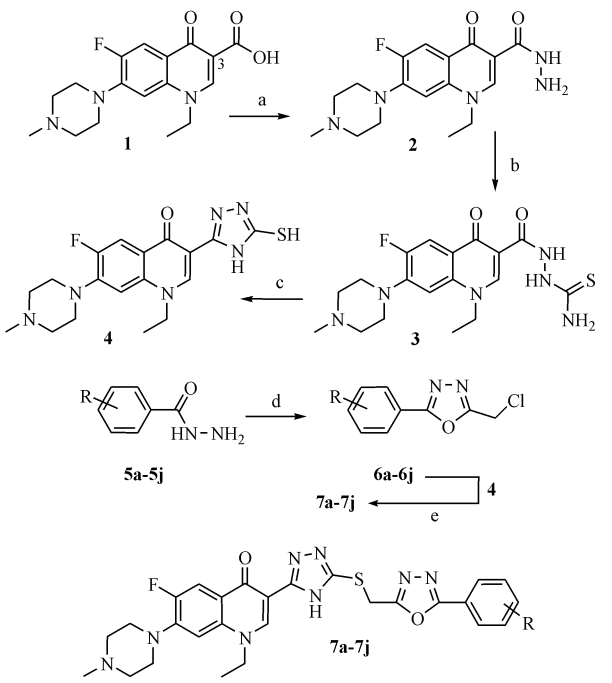
为修饰杂环,设计合成了10个未见报道的C-3均三唑噁二唑甲硫醚衍生物(**7a**~**7j**,表1),并评价了其体外的抗肿瘤活性。

1 合成路线

目标化合物**7a**~**7j**的制备见路线1。培氟沙星酰肼**2**由培氟沙星**1**羧酸直接肼化而得[3]。2-芳基-[1,3,4]-噁二唑-5-氯甲烷**6a**~**6j**由相应的芳酰肼**5a**~**5j**与氯乙酸在三氯氧磷和甲苯中环合而得。上述产生的中间体**4**和**6**在氢氧化钠-乙醇介质中发生亲核取代反应到硫醚目标产物**7a**~**7j**。



7a R=H **7f** R=4-CH_3
7b R=4-OCH_3 **7g** R=3-CH_3
7c R=2-OCH_3 **7h** R=4-Cl
7d R=3,4-$(OCH_3)_2$ **7i** R=4-F
7e R=3,4,5-$(OCH_3)_3$ **7j** R=4-O_2N

Scheme 1 Synthetic route of the target compounds **7a-7j**

a:80% $N_2H_4H_2O$, reflux; b: KSCN, HCl-H_2O, reflux; c: NaOH-H_2O, reflux; d: $ClCH_2COOH$, $POCl_3$, $PhCH_3$, reflux; e: EtOH, NaOH, r. t.

2 实验部分

2.1 材 料

上海申光WK-1B数字熔点仪;德国Bruker公司Esquire LC型质谱仪;Bruker AM-400型核磁共振仪测定;美国Nicolet Avatar 360红外光谱仪,KBr压片;美国PE PE2400-Ⅱ元素分析仪。培氟沙星**1**为市售品,培氟沙星酰肼**2**[7]及中间体**6a**~**6j**[4]分别按文献的方法制备,试剂均为市售分析纯。

2.2 化学合成

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-(5-巯基-4*H*-[1,2,4]-三唑-3-基)-喹啉(1*H*)-4-酮(**4**)

培氟沙星酰肼**2**(10.0 g,29.0 mmol)溶于6%盐酸水溶液(150 mL)中,加入硫氰化钾(4.2 g,43.0 mmol)回流反应12 h。滤集产生的固体,加入到6%氢氧化钠水溶液(150 mL)中,回流反应10 h。用适量活性炭脱色,滤液用盐酸中和至pH 7.0,滤集产生的固体,水洗,干燥。用DMF重结晶,得淡黄色固体**4**,收率68.0%,mp:224~226 ℃;^{1}H NMR δ:13.62,13.78(s,2H,SH,NH),8.73(s,1H,2-H),7.74(s,1H,5-H),7.24(s,1H,8-H),4.48(q,J=7.1 Hz,2H,$C\underline{H}_2CH_3$),3.53~2.87(m,8H,piperazine-H),2.62(s,3H,N-CH_3),1.34(t,J=7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 368,1 634,1 457,1 268 cm^{-1};MS m/z 389$[M+H]^+$。

2.3 1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[5-(5-芳基-[1,3,4]-噁二唑-2-甲硫基)-4*H*-[1,2,4]-三唑-3-基]-喹啉(1*H*)-4-酮(**7a**~**7j**)合成通法

中间体**4**(0.5 g)悬浮于乙醇(20 mL)中,冰浴下用40%的氢氧化钠水溶液调pH 8.0~9.0,加入等物质的量的氯甲基噁二唑中间体**6**,保温维持弱碱性下反应至原料消失。用冰醋酸调至弱酸性,减压蒸除溶剂,加水(20 mL)和适量活性炭回流脱色30 min。滤液用氨水碱化,滤集产生的固体,水洗,干燥。粗品用无水乙醇-DMF重结晶,得淡黄色固体目标化合物**7a**~**7j**。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-[5-(5-苯基-[1,3,4]-噁二唑-2-甲硫基)-4*H*-[1,2,4]-三唑-3-基]-喹啉(1*H*)-4-酮(**7a**),收率87%,mp:189~191 ℃;^{1}H NMR δ:13.34(s,1H,NH),8.84(s,1H,2-H),7.86~7.27(m,7H,5-H,8-H,Ph-H),4.68(s,2H,SCH_2),4.42(q,J=7.1 Hz,2H,$C\underline{H}_2CH_3$),3.56~3.17(m,8H,piperazine-H),2.64(s,3H,N-CH_3),1.35(t,J=7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 457,1 638,1 455,1 267 cm^{-1};MS m/z 547$[M+H]^+$;Anal. calcd. for $C_{27}H_{27}FN_8O_2S$: C 59.33, H 4.98, N 20.50; Found: C 59.53, H 4.76, N 20.74。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(对甲氧苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7b**),收率86%,mp:192~193 ℃;^{1}H NMR δ:13.36(s,1H,NH),8.87(s,1H,2-H),7.88(d,J=13.2 Hz,1H,5-H),7.76(d,J=7.4 Hz,2H,Ph-H),7.62(d,J=7.4 Hz,2H,Ph-H),7.26(d,J=7.2 Hz,1H,8-H),4.72(s,2H,SCH_2),4.48(q,J=7.1 Hz,2H,CH_2),3.87(s,3H,OCH_3),3.57~3.23(m,8H,piperazine-H),2.66(s,3H,N-CH_3),1.34(t,J=7.1 Hz,3H,CH_3);IR(KBr,υ):3 468,1 642,1 467,1 268 cm^{-1};MS m/z 577$[M+H]^+$;Anal.

calcd. for $C_{28}H_{29}FN_8O_3S$: C 58.32, H 5.07, N 19.43; Found: C 58.48, H 4.74, N 19.68。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(邻甲氧苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7c**),收率 72%,mp:172 ~ 174 ℃;^{1}H NMR δ:13.38(s,1H,NH),8.89(s,1H,2-H),7.96(s,1H,5-H),7.88 ~ 7.64(m,4H,Ph-H),7.28(d,1H,8-H),4.74(s,2H,SCH_2),4.47(s,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_3$),3.86(s,3H,OCH_3),3.55 ~ 3.27(m,8H,piperazine-H),2.63(s,3H,N-CH_3),1.35(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 368,1 637,1 557,1 326 cm^{-1};MS *m/z* 577[M + H]$^+$;Anal. calcd. for $C_{28}H_{29}FN_8O_3S$: C 58.32, H 5.07, N 19.43; Found: C 58.45, H 4.84, N 19.62。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(3,4-二甲氧苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7d**),收率 68%,mp:174 ~ 176 ℃;^{1}H NMR δ:13.35(s,1H,NH),8.87(s,1H,2-H),7.86(s,1H,5-H),7.78 ~ 7.62(m,3H,Ph-H),7.26(s,1H,8-H),4.68(s,2H,SCH_2),4.45(q,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_3$),3.87 and 3.88(s,6H,2 × OCH_3),3.52 ~ 3.05(m,8H,piperazine-H),2.58(s,3H,N-CH_3),1.32(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 357,1 634,1 546,1 324 cm^{-1};MS *m/z* 607[M + H]$^+$;Anal. calcd. for $C_{29}H_{31}FN_8O_4S$: C 57.41, H 5.15, N 18.47; Found: C 57.66, H 5.02, N 18.29。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(3,4,5-三甲氧苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7e**),收率 75%,mp:168 ~ 170 ℃;^{1}H NMR δ:13.37(s,1H,NH),8.87(s,1H,2-H),8.12(s,2H,Ph-H),7.87(s,1H,5-H),7.25(s,1H,8-H),4.74(s,2H,SCH_2),4.47(q,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_3$),3.86,3.92(s,9H,3 × OCH_3),3.54 ~ 3.16(m,8H,piperazine-H),2.62(s,3H,N-CH_3),1.34(t,*J* = 7.1 Hz,3H,$C\underline{H}_2CH_3$);IR(KBr,υ):3 367,1 636,1 558,1 326 cm^{-1};MS *m/z* 637[M + H]$^+$;Anal. calcd. for $C_{30}H_{33}FN_8O_5S$: C 56.59, H 5.22, N 17.60; Found: C 56.82, H 5.47, N 17.83。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(对甲苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7f**),收率 81%,mp:158 ~ 160 ℃;^{1}H NMR δ:13.26(s,1H,NH),8.84(s,1H,2-H),7.87(s,1H,5-H),7.74(d,*J* = 7.4 Hz,2H,Ph-H),7.58(d,*J* = 7.4 Hz,2H,Ph-H),7.23(s,1H,8-H),4.68(s,2H,SCH_2),4.46(q,*J* = 7.1 Hz,2H,CH_2),3.52 ~ 3.08(m,8H,piperazine- H),2.62(s,3H,N-CH_3),2.42(s,3H,Ph-CH_3),1.32(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 358,1 632,1 557,1 287 cm^{-1};MS *m/z* 561[M + H]$^+$;Anal. calcd. for $C_{28}H_{29}FN_8O_2S$: C 59.99, H 5.21, N 19.99; Found: C 60.22, H 4.94, N 20.26。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(间甲苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7g**),收率 62%,mp:156 ~ 158 ℃;^{1}H NMR δ:13.31(s,1H,NH),8.86(s,1H,2-H),7.86(S,1H,5-H),7.76 ~ 7.60(m,4H,Ph-H),7.22(S,1H,8-H),4.68(s,2H,SCH_2),4.47(q,*J* = 7.1 Hz,2H,CH_2),3.48 ~ 3.07(m,8H,piperazine- H),2.58(s,3H,N-CH_3),2.37(s,3H,Ph- CH_3),1.33(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 348,1 628,1 546,1 268 cm^{-1};MS *m/z* 561[M + H]$^+$;Anal. calcd. for $C_{28}H_{29}FN_8O_2S$: C 59.99, H 5.21, N 19.99; Found: C 60.20, H 4.98, N 20.23。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(对氯苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7h**),收率 84%,mp:184 ~ 186 ℃;^{1}H NMR δ:13.37(s,1H,NH),8.92(s,1H,2-H),8.16(d,*J* = 7.5 Hz,2H,Ph-H),7.89(S,1H,5-H),7.82(d,*J* = 7.5 Hz,2H,Ph-H),7.34(s,1H,8-H),4.76(s,2H,SCH_2),4.53(q,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_3$),3.53 ~ 3.16(m,8H,piperazine-H),2.62(s,3H,N-CH_3),1.37(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 358,1 644,1 562,1 346 cm^{-1};MS *m/z* 581[M + H]$^+$;Anal. calcd. for $C_{27}H_{26}ClFN_8O_2S$: C 55.81, H 4.51, N 19.28; Found: C 56.04, H 4.27, N 19.53。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(对氟苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7i**),收率 86%,mp:214 ~ 216 ℃;^{1}H NMR δ:13.38(s,1H,NH),8.96(s,1H,2-H),8.24(d,*J* = 7.5 Hz,2H,Ph-H),8.15(s,1H,5-H),7.88(d,*J* = 7.5 Hz,2H,Ph-H),7.36(s,1H,8-H),4.78(s,2H,SCH_2),4.54(q,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_2$),3.57 ~ 3.20(m,8H,piperazine-H),2.64(s,3H,N-CH_3),1.38(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 364,1 646,1 567,1 327 cm^{-1};MS *m/z* 565[M + H]$^+$;Anal. calcd. for $C_{27}H_{26}F_2N_8O_2S$: C 57.44, H 4.64, N 19.85; Found: C 57.67, H 4.41, N 20.06。

1-乙基-6-氟-7-(4-甲基哌嗪-1-基)-3-{5-[5-(对硝基苯基)-[1,3,4]-噁二唑-2-甲硫基]-4*H*-[1,2,4]-三唑-3-基}-喹啉(1*H*)-4-酮(**7j**),收率 72%,mp:228 ~ 230 ℃;^{1}H NMR δ:13.42(s,1H,NH),9.15(s,1H,2-H),8.36(d,*J* = 7.5 Hz,2H,Ph-H),8.25(s,1H,5-H),8.12(d,*J* = 7.5 Hz,2H,Ph-H),7.40(s,1H,8-H),4.82(s,2H,SCH_2),4.58(q,*J* = 7.1 Hz,2H,$C\underline{H}_2CH_3$),3.56 ~ 3.25(m,8H,piperazine-H),2.66(s,3H,N-CH_3),1.40(t,*J* = 7.1 Hz,3H,$CH_2C\underline{H}_3$);IR(KBr,υ):3 451,1 648,1 557,1 342 cm^{-1};MS *m/z* 592[M + H]$^+$;Anal. calcd. for $C_{27}H_{26}FN_9O_4S$: C 54.81, H 4.43, N 21.31; Found: C 54.98, H 4.26, N 21.54。

2.3　抗肿瘤活性评价

对合成的 10 个双杂环硫醚目标化合物 **7a** ~ **7j**

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稀释到所需浓度。取对数生长期的中国仓鼠卵巢(CHO)细胞以每孔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,每孔加入二甲基亚砜150 μL,轻微振荡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7 000个细胞接种于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,计算各组对肿瘤细胞的抑制率:细胞抑制率=(1-实验组吸收度/对照组吸收度)×100%。然后以各药物浓度对数值对各浓度下的抑制率作线性回归得剂量-效应方程,依此计算出各供试化合物对实验肿瘤细胞的半数抑制浓度(IC_{50}),结果见表1。

Table 1 Inhibitory activities of the target compounds(**7a-7j**) against L1210, HL60 and CHO tumor cells

Compd.	IC_{50}/(μmol/L)		
	L1210	HL60	CHO
7a	12.7	15.3	10.3
7b	14.6	17.4	12.7
7c	5.6	7.6	6.4
7d	15.7	17.2	12.1
7e	20.6	24.5	16.8
7f	26.3	32.4	24.6
7g	18.5	23.4	15.6
7h	20.7	25.7	18.4
7i	4.7	6.2	3.8
7j	30.5	35.2	27.4
Pefloxacin	>150	>150	>150

体外抗肿瘤筛选结果(表1)表明,10个目标化合物对3种试验肿瘤细胞株的半数抑制浓度均低于35.0 μmol/L,显著强于对照培氟沙星的活性(>150 μmol/L),表明羧基并非抗肿瘤的必要基团,可被杂环等排体替代。对修饰杂环噁二唑的取代基而言,随着体积的增大,活性降低;供电子基取代的活性高于吸电子基的活性,这为氟喹诺酮向抗肿瘤活性的转化提供了有效途径。

参考文献

[1] Trippier PC, Jansen Labby K, Hawker DD, *et al*. Target- and mechanism-based therapeutics for neurodegenerative diseases: strength in numbers[J]. *J Med Chem*, 2013, **56**(8): 3 121-3 147.

[2] Bax BD, Chan PF, Eggleston DS, *et al*. Type IIA topoisomerase inhibition by a new class of antibacterial agents[J]. *Nature*, 2010, **466**(7 309): 935-940.

[3] Xu QJ, Hou LL, Wu XK, *et al*. Synthesis and antitumor and antibacterial activity of ciprofloquinolone bis-(C/C7hydrazone)s[J]. *J China Pharm Univ*(中国药科大学学报), 2013, **44**(1): 35-38.

[4] Hu GQ, Hou LL, Wang GQ, *et al*. Design, synthesis and antitumor activity of fluoroquinolone C3 heterocycles: bis-oxadiazole methylsulfide derivatives derived from ciprofloxacin[J]. *Acta Pharm Sin*(药学学报), 2012, **47**(8): 1 017-1 022.

[5] Zhou CH, Wang Y. Recent researches in triazole compounds as medicinal drugs[J]. *Curr Med Chem*, 2012, **19**(2): 239-280.

[6] Du QR, Li DD, Pi YZ, *et al*. Novel 1,3,4-oxadiazole thioether derivatives targeting thymidylate synthase as dual anticancer/antimicrobial agents[J]. *Bioorg Med Chem*, 2013, **21**(8): 2 286-2 297.

[7] Wang GQ, Duan NN, Cao TY, *et al*. Antitumor fluoroquinolone C-3-isostere derivatives(Ⅰ): synthesis and activity of bis-oxadiazole methyl-sulfide derivatives[J]. *Chin J Appl Chem*(应用化学), 2012, **29**(7): 769-774.