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3-FLUORO-2-(4-METHYLPIPERAZIN-1-YL)-5,12-DIHYDRO-5-OXOBENZOXAZOLO[3,2-*a*]QUINOLINE-6-CARBOXYLIC ACID: SYNTHESIS AND *In vitro* CYTOTOXIC ACTIVITY

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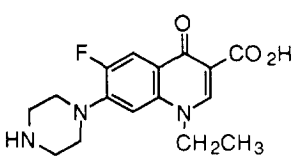
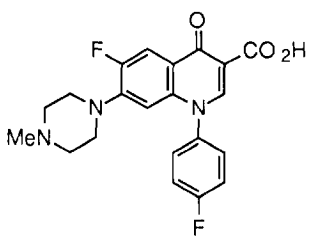
Abstract : The title compound which shows a potent *in vitro* cytotoxic activity has been synthesized employing the tandem double ring closure reaction of *N*-acyl-*N*-(2'-hydroxyphenyl) anthranilic acid with acetic anhydride as a key step.

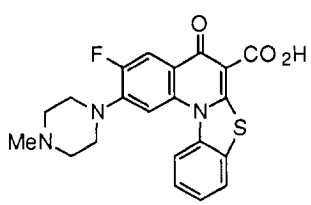
DNA topoisomerases are receiving an increasing attention. These enzymes play a critical role in DNA replication and transcription by altering its topological state.[1] Of these, bacterial DNA topoisomerase II (DNA gyrase) has been of particular interest to medicinal chemists because inhibition of this enzymic action leads to selective intoxication of bacteria.[2] The antibacterial property of the quinolones is attributed to this selective toxic effect. Recently, it has also been shown that some quinolones inhibit mammalian topoisomerase II as well to manifest cytotoxic activity, and thus quinolones have become viable lead compounds in the development of cancer chemotherapeutic agents.[3]



norfloxacin



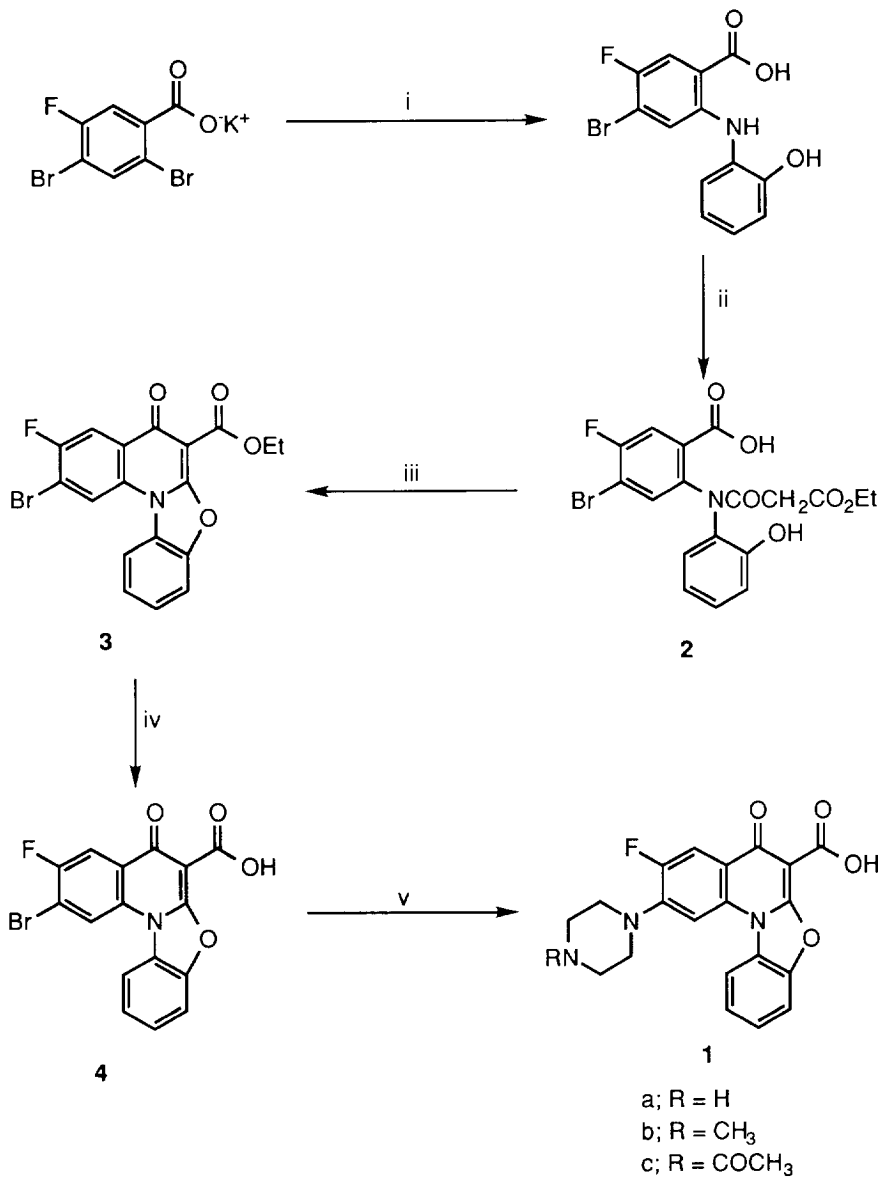
difloxacin



A-57,207

Chu et al.[4] reported that in fluoroquinolones[5] the small alkyl group at the 1-position, whose presence was thought to be essential for antibacterial activity, may be replaced with an aryl group with retention of the biological activity, as exemplified by difloxacin and A-57,207. In this communication, we wish to report the successful synthesis of 3-fluoro-2-(4-methylpiperazin-1-yl)-

Scheme 1.



Reagents and conditions: i) 2-aminophenol (2.5 eq.), $CuCl_2$ (cat.), n-BuOH, 2 h, reflux (yield: 19%); ii) ethyl malonyl chloride, imidazole (3 eq.), CH_2Cl_2; iii) Ac_2O, 80°C, 3 h (yield of ii and iii: 60%); iv) sulfuric acid (95%), 8 h, 70°C (yield: 91%); v) 1-methylpiperazine (4 eq.), 1-methyl-2-pyrrolidinone, 110°C, 1.5 h (yield: 52%)

5,12-dihydro-5-oxobenzoxazolo[3,2-*a*]quinoline-6-carboxylic acid (**1b**),[6] the oxygen isostere of A-57,207, and related compounds and their biological activity.

The synthesis[7] makes use of the tandem double ring closure reaction of *N*-(2'-hydroxyphenyl)-anthranilic acid with acetic anhydride as a key step.[8] 4-Bromo-5-fluoro-*N*-(2'-hydroxyphenyl)-anthranilic acid that was prepared from 2,4-dibromo-5-fluorobenzoic acid following the literature method[8] was allowed to react with ethyl malonyl chloride in the presence of imidazole (3 eq.) to give **2**. The key step of the synthesis to prepare **3** was achieved in a good yield (90%) by simply treating **2** with acetic anhydride at 80 ℃. Hydrolysis of the ester moiety in **3** was achieved smoothly by the treating with concentrated sulfuric acid to give the corresponding acid **4**. The introduction of the piperazine moiety into the 2-position of **4** was carried out by heating **4** with an excess of 1-methylpiperazine in 1-methyl-2-pyrrolidinone at 110 ℃ to give **1b** (Scheme 1).[9] Similarly prepared were **1a** and **1c**.

Table 1. *In vitro* IC_{50} values[a] (μg/ml) against human tumor cell lines

Compound	Human Tumor Cell Lines		
	SNU 1 (Stomach)	SNU 354 (Liver)	SNU C-4 (Colon)
1a	15.3	19.8	13.4
1b·HCl	2.0	1.9	1.8
1c	>200	>200	>200
Doxorubicin	<0.5	<0.5	<0.5
cis-Platinum	1.4	4.1	2.8

[a] *In vitro* cytotoxic activities were examined by the modified SRB assay method.[12]

To our surprise, neither **1** nor its analogs showed any significant antibacterial activity when assayed by the agar dilution method.[10] The reason for the lack of antimicrobial activity is not apparent to us, but we suspect the planar molecular conformation of **1** and the increased electrophilic property of the oxazole ring compared with the thiazole in A-57,207 as possible causes. The PM3 calculations showed that while the phenyl ring of benzothiazolo[3,2-*a*]quinolone orients out of the plane of quinolone nucleus having a dihedral angle of 23°, the two rings in benzoxazolo[3,2-*a*]quinolone are oriented having a near coplanar conformation. Recently, Ohta and Koga[11] suggested that in order for 1-phenylquinolone to have an antibacterial activity, the phenyl ring should be oriented perpendicular to the quinolone ring with a dihedral angle of 110°. However, as shown in Table 1, these compounds showed *in vitro* cytotoxic activity[12] with the potency slightly less than that of adriamycin but more potent than cisplatin when evaluated against several human tumor cell lines[13]. The observation is worth noting in view of the current thought that the cytotoxic effect and antibacterial activity of quinolones usually go parallel, *i.e.* the most cytotoxic quinolones are also the best antibacterial agents.[14] We are exploring the lead for development of therapeutically useful antitumor agents.

References and Notes

1. (a) Drlica, K. *Microbiol. Rev.* **1984**, *48*, 273-289.; (b) Gellert, M. *Annu. Rev. Biochem.* **1981**, *50*, 879-910.; (c) Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665-697.; (d) Wang, J. C. *Biochim. Biophys. Acta* **1985**, *909*, 1-9.
2. (a) O'Coner, M. B.; Malamy, M. H. *J. Mol. Biol.* **1985**, *181*, 545-550.; (b) Crumplin, G. C.; Kenwright, M.; Hirst, T. *J. Antimicrob. Chemother.* **1984**, *13* (Suppl. B), 9-23.; (c) Shen, L. L.; Mitscher, L. A.; Shamara, P. N.; O'Donnell, T. J.; Chu, D. T. W.; Cooper, C. S.; Rosen, T.; Pernet, A. G. *Biochemistry*, **1989**, *28*, 3886-3894.
3. (a) Robinson, M. J.; Martin, B. A.; Gootz, T. D.; McGuirk, P. R.; Moynihan, M.; Sutcliffe, J. A.; Osheroff, N. *J. Biol. Chem.* **1991**, *266*, 14585-14592.; (b) Elsea, S. H.; Osheroff, N.; Nitiss, J. L. *J. Biol. Chem.* **1992**, *267*, 13150-13153.; (c) Wentland, M. P.; Lesher, G. Y.; Reuman, M.; Gruett, M. D.; Singh, B.; Aldous, S. C.; Dorff, P. H.; Rake, J. B.; Coughlin, S. A. *J. Med. Chem.* **1993**, *36*, 2801-2809.; (d) Yamashita, Y.; Ashizawa, T.; Morimoto, M.; Hosomi, J.; Nakano, H. *Cancer Res.* **1992**, *52*, 2818-2822.
4. (a) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nardeen, C. W.; Maleczka, R. E., Jr.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558-1564.; (b) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, E. H.; Pernet, A. G. *J. Med. Chem.* **1986**, *29*, 2363-2369.
5. (a) Paton, J. H.; Reeves, D. S. *Drugs* **1988**, *36*, 193-228.; (b) *The New Generation of Quinolones;* Simporin, C.; Heitz, C. L.; Domagala, J. M. Eds.; Marcel Deckker: New York, 1990.
6. Chu (South African Patent 852, 802 (1995)) claimed the synthesis of **1b** starting with condensation of 2,4-dichloro-5-fluorobenzoylacetic acid trimethylsilylethyl ester with 2-chlorobenzoxazole in the presence of sodium hydride at 160 °C to form 2-chloro-3-fluoro-5,12-dihydro-5-oxobenzoxazole[3,2-*a*]quinoline-6-carboxylic acid trimethylsilylethyl ester, and subsequent hydrolysis followed by dechloroamination with 1-methylpiperazine, but we were unable to reproduce the condensation reaction. Furthermore, no physical and spectral data were provided for the claimed compound and its precursors.
7. The structure of all new compounds described in this communication are supported by spectral data as well as by satisfactory results of elemental analysis.
8. (a) Kim, D. H.; Fieber, R. A.; Santilli, A. A.; Bell, S. C. *J. Heterocyclic Chem.* **1974**, *11*, 703-706.; (b) Kim, D. H. *J. Heterocyclic Chem.* **1981**, *18*, 1389-1392.
9. **1a**: Mp: >280 °C IR (KBr) cm^{-1}: 3500-2500, 3219 (NH), 1725, 1630; ^1H NMR (TFA-d): δ 3.85 (4H, s), 4.10 (4H, s), 7.95 (2H, m), 8.11 (1H, d), 8.15 (1H, d), 8.48 (2H, m); Anal. calcd. for $C_{20}H_{16}FN_3O_4 \cdot 1/2H_2O$: C 61.53, H 4.39, N 10.77. Found: C 61.85, H 4.06, N 10.52. **1b**: Mp: >280 °C IR (KBr) cm^{-1}: 3452, 1732, 1631; ^1H NMR (TFA-d): δ 8.42-8.50 (2H, t), 8.19 (1H, d), 8.10 (1H, d), 7.93-8.00 (2H, m), 4.34 (2H, d), 3.99 (2H, d), 3.65 (2H, t), 3.60 (2H, t), 3.24 (3H, s); EIMS *m/z* 395 (M^+), 351; Anal. calcd. for $C_{21}H_{18}FN_3O_4$: C 63.79, H 4.59, N 10.63. Found: C 63.59, H 4.43, N 10.42. **1c**: Mp: >280 °C IR (KBr) cm^{-1}: 3500-2500, 1717, 1630; ^1H NMR (TFA-d): δ 2.48 (3H, s), 3.87-3.95 (4H, d), 4.00-4.20 (4H, d), 7.80-7.91 (3H, m), 8.06 (1H, d), 8.30-8.40 (2H, t); Anal. calcd. for $C_{22}H_{18}FN_3O_5 \cdot 1/3H_2O$: C 61.54, H 4.38, N 9.79. Found: C 61.82, H 4.27, N 9.68.
10. Chu, D. T. W.; Nordeen, C. W.; Hardy, D. J.; Swanson, R. N.; Giardina, W. J.; Pernet, A. G.; Plattner, J. J. *J. Med. Chem.* **1991**, *34*, 168-174.
11. Ohta, M.; Koga, H. *J. Med. Chem.* **1991**, *34*, 131-139.
12. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107-1112.
13. (a) Park, J.; Frucht, H.; LaRocca, R. V.; Bliss, D. P., Jr.; Kurita, Y.; Chen, T.-R.; Henslee, J. G.; Trepel, J. B.; Jensen, R. T.; Johnson, B. E.; Bang, Y.-J.; Kim, J.-P.; Gazdar, A. F. *Cancer Res.* **1990**, *50*, 2773-2780; (b) Park, J.; Oie, H. K.; Sugarbaker, P. H.; Henslee, J. G.; Chen, T.; Johnson, B. E.; Gazdar, A. F. *Cancer Res.* **1987**, *47*, 6710-6718.
14. (a) Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A. *J. Med. Chem.* **1992**, *35*, 4745-4750.; (b) Chu, D. T. W. *Drugs of the Future* **1992**, *17*, 1101-1109.

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