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ARTICLE *in* PHARMACEUTICAL CHEMISTRY JOURNAL · JULY 2011

Impact Factor: 0.45 · DOI: 10.1007/s11094-011-0595-x

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Pharmaceutical Chemistry Journal, Vol. 45, No. 4, July, 2011 (Russian Original Vol. 45, No. 4, April, 2011)

SYNTHESIS AND ANTITUMOR ACTIVITY OF FLUORINATED DERIVATIVES OF [*i,j*]-ANNELATED QUINOLONES

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 45, No. 4, pp. 12 – 14, April, 2011.

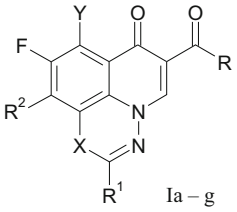
Original article submitted December 29, 2009.

Tri- and pentacyclic fluoroquinolones were synthesized by intramolecular cyclization of the corresponding 3-hydrazinopolyfluorobenzoylacrylates followed by substitution of fluorine atoms by amine residues. The antitumor activity of the resulting compounds was studied at the National Cancer Institute using cultures of 60 cell lines of nine groups, including leukemia, lung tumor, large intestine tumor, CNS tumor, melanoma, ovary tumor, renal tumor, prostate tumor, and breast tumor. Relationships between structure and antitumor activity were analyzed. In vivo experimental data from hollow fiber tests are presented for two derivatives.

Key words: 1,3,4-Thiadiazino[5,6,4-*i,j*]quinoline, benzimidazo[2′,3′:3,4]-1,2,4-triazino[5,6,1-*i,j*]quinoline, synthesis, antitumor activity.

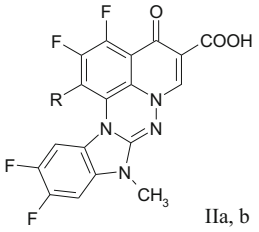
We have previously reported the synthesis of polycyclic analogs of fluoroquinolone antibiotics [1 – 3] and the finding that some derivatives of this series have antitumor activity [4]. In continuation of our studies on the synthesis and antitumor properties of [*i,j*]-annelated fluoroquinolone carboxylic acids we have obtained new derivatives of 1,3,4-thiadiazino[5,6,4-*i,j*]quinoline (I) and benzimidazo-[2′,3′:3,4]-[1,2,4]triazino[5,6,1-*i,j*]quinoline (II).

Compounds I and II were prepared by intramolecular cyclization of the corresponding 3-hydrazinopolyfluorobenzoylacrylates followed by substitution of fluorine atoms by amine residues. The synthesis of compounds Ia-g and IIb has been described in detail elsewhere [2, 3, 5 – 7]. In the present report, we will present a method for the synthesis of the previously undescribed derivative IIa.



Ia – g

a: X = S, R = OEt, R^1 = pyrrolidin-1-yl;
R^2 = Y = N,N-dimethyl-1,3-diaminopropane;
b: X = S, R = OEt, R^1 = pyrrolidin-1-yl; R^2 = Y = F;
c: X = S, R = OEt, R^1 = cyclohexylamine, R^2 = Y = F;
d: X = S, R = OH, R^1 = cyclohexylamine, R^2 = F, Y = H;
e: X = S, R = OEt, R^1 = cyclohexylamine, R^2 = F, Y = H;
f: X = O, R = OEt, R^1 = pyridin-3-yl; R^2 = Y = F;
g: X = O, R = OEt, R^1 = nitrophenyl, R^2 = Y = F.



IIa, b

a: R = pyrrolidin-1-yl;
b: R = F.

EXPERIMENTAL CHEMICAL SECTION

1-(Pyrrolidin-1-yl)-2,3,10,11-tetrafluoro-8-methyl-4-oxo-4H-benzimidazo[2′,3′:3,4]-1,2,4-triazino[5,6,1-*i,j*]quinolin-5-carboxylic acid (IIa). A suspension of 0.65 g (1.48 mmol) of 1,2,3,10,11-pentafluoro-8-methyl-4-oxo-4H-benzimidazo[2′,3′:3,4]-1,2,4-triazino[5,6,1-*i,j*]quinolin-5-carboxylic acid ethyl ester in 10 ml of pyridine was supplemented with 0.38 ml (4.43 mmol) of pyrrolidine and the reaction mix was boiled for 5 h. The precipitate of 1-(pyrrolidin-1-yl)-2,3,10,11-tetrafluoro-8-methyl-4-oxo-4H-benzi

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TABLE 1. Antitumor Activity of Compounds Ia, b,f, g and IIa, b (at a Concentration of 10^{-4} M) against Three Cell Lines.

Compound	NCI-H460 (lung tumor)	MCF7 (breast tumor)	SF-268 (CNS tumor)
Ia	– 71	– 91	– 95
Ib	– 45	– 86	– 30
If	– 73	– 33	– 11
Ig	– 62	– 87	– 50
IIa	– 86	– 62	– 92
IIb	– 65	– 97	– 77

midazo[2′,3′:3,4]-1,2,4-triazino[5,6,1-*i,j*]quinolin-5-carboxy lic acid ethyl ester forming on cooling was collected by filtration, washed with ether, and recrystallized from DMSO. A solution of 0.5 g (1.0 mmol) of the resulting 1-pyrrolidino-substituted ethyl ester in 20 ml of a mixture of hydrochloric and acetic acids (1:4) was boiled for 3 h. After cooling and dilution with water, the carboxylic acid (IIa) precipitate was collected by filtration and recrystallized from dimethylformamide. The yield was 0.4 g (60%). T_m was 262 – 264°C. $C_{22}H_{15}F_4N_3O_3$. The ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) (SSCC, Hz), was: 1.90 (m, 4H, $(CH_2)_2$), 3.36 (m, 4H, $N(CH_2)_2$), 3.41 (s, 3H, NCH_3),7.43(dd, 1H, H^{12}, $^3J(H^{12}, F_{11})$ 11.3, $^4J(H^{12}, F^{10})$, 7.2); 7.45 (dd, 1H, H^9, F^{10}) 11.3, $^4J(H^9, F^{11})$ 7.2), 8.16 (s, 1H, H^6), 15.4 (broad s, 1H, COOH). The ^{19}F NMR spectrum (DMSO-d_6), δ_F, ppm (SSCC, Hz), was: 135.05 (d, 1F, F^3, $^3J(F^3, F^2)$ 19.6), 143.12 (dddd, 1F, F^{11}, $^3J(F^{11}, F^{10})$ 21.3, $^3J(F^{11}, H^{12})$ 10.7, $^4J(F^{11}, H^9)$ 6.9, $^9J(F^{11}, H^{5'})$ 3.3), 145.92 [d.d.d, 1F, F^{10}, $^3J(F^{10}, F^{11})$ 21.3, $^3J(F^{10}, H^9)$ 11.4, $^4J(F^{10}, H^{12})$ 7.2); 146.06 (d.t, 1F, F^2, $^3J(F^2, F^3)$ 19.6, $^5J(F^2, H^{2'})$ 2.5). The mass spectrum, *m/z* (I_{rel}), was: 473 ([M^+], 28), 453 (21), 407 (29), 387 (21), 386 (86), 360 (100), 345 (72), 305 (16), 228 (21).

TABLE 2. Antitumor Activity of Pentacyclic (IIa, b) and Tricyclic (If, g) Quinolone Derivatives.

Line	Value lg(GI_{50})			
	Ia	Ib	If	Ig
Leukemia	– 5.91	– 6.08	– 5.31	– 4.62
Lung tumor (non-small cell)	– 5.73	– 5.66	– 4.73	– 4.68
Large intestine tumor	– 5.71	– 6.14	– 4.94	– 4.72
CNS tumor	– 5.65	– 5.51	– 4.74	– 4.63
Melanoma	– 5.71	– 5.85	– 4.83	– 4.73
Ovary tumor	– 5.67	– 5.67	– 4.64	– 4.59
Kidney tumor	– 5.83	– 5.76	– 4.79	– 4.67
Prostate tumor	– 5.71	– 5.73	– 4.90	– 4.83
Breast tumor	– 5.70	– 5.80	– 4.82	– 4.66
Mean lg(GI_{50})	– 5.73	– 5.81	– 4.84	– 4.67
Mean lg(TG_1)	– 5.13	– 5.23	– 4.28	– 4.31
Mean lg(LC_{50})	– 4.48	– 4.67	– 4.03	– 4.08

TABLE 3. Antitumor Activity of Compounds Ic-e (at a Concentration of 10 μg/ml) against Three Cell Lines.

Compound	A549 (lung tumor)	SK-Mel-2 (melanoma)	SK-OV-3 (ovary tumor)
Ic	– 10	– 20	– 90
Id	75	5	– 55
Ie	5	– 20	– 75

EXPERIMENTAL BIOLOGICAL SECTION

Studies of the antitumor activity of compounds I and II were performed on cultures of 60 tumor cell lines of nine groups, including leukemia, lung tumor, large intestine tumor, CNS tumor, melanoma, ovary tumor, renal tumor, prostate tumor, and breast tumor. Compounds were tested at five concentrations obtained by ten-fold dilutions. Exposure was for 48 h. Cell viability was assessed by staining protein with sulforhodamine B (SRB); optical densities for each concentration for each cell line were measured experimentally. Optical density values were used to calculate percentage growth. Results are presented as percentages over the range +100% to –100%, where values from 0 to +100 designate only inhibition of cell growth, while values of 0 to -100 indicate cell death. The level of cell growth suppression was assessed in terms of the parameters GI_{50} (an interpolated value corresponding to the concentration at which cell growth was inhibited by 50%), TG_1 (complete suppression of cell growth), and LC_{50} (50% cell death).

For compounds Ic – e, in vitro antitumor tests were performed at the Samsung Institute of Technology using three tumor cell lines: A549 (lung tumor), SKOV-3 (ovary tumor), and SK-MEL-2 (melanoma) at four concentrations (0.01, 0.1, 1, and 10 mg/ml).

In vitro test results were used to select compounds Ib and IIb for further testing in in vivo experiments. Four experiments were performed, each of which included tests on three tumor cell lines; a total of 12 human tumor cell lines were tested. In this test, human tumor cells were cultured in polyvinylidene fluoride fibers and samples of each cell line were implanted i.p. and s.c. Each mouse received a total of six fibers (three i.p. and three s.c.) with three different tumor

TABLE 4. Antitumor Activity of Compounds IIb and Ib *in vivo*, Hollow Fiber Method.

Test data	Compound	
	IIb	Ib
IP points (i.p. administration)	10	4
SC points (s.c. administration)	2	2
Total IP + SC points	12	6
Cell death seen	yes	no

cell line. Groups of three mice received compound Ib or compound IIb i.p. at two doses. Six control mice received only solvent. Fiber cultures were collected on the day following the last doses. Antitumor effects were assessed by measuring the viable cellular mass for each cell line by formazan stain transformation (MTT). I.p. and s.c. samples were assessed separately. The National Cancer Institute uses a system of points in which 2 scores are assigned to each dose reducing the viable mass by 50% or more.

RESULTS AND DISCUSSION

Analysis of the data presented in Tables 1 and 2 showed that among compounds I, substitution of the fluorine atom by the pharmacophore fragment N,N-dimethyl-1,3-diaminopropane (compounds Ia and Ib, Table 1) had the greatest influence on antitumor activity. Compound Ia produced virtually complete death (more than 90%) of MCF7 and SF-268 tumor cells. Substituents had significantly less effect on antitumor activity in compounds II. Thus, introduction of a pyrrolidine fragment at position 1 (compound IIa) led to an increase in percentage death of only two tumor cell lines – NCI-H460 and SF-268 (Table 1).

Compound IIa was studied in cultures of 60 tumor cell lines in nine groups; these experiments showed that there was no significant increase in activity as compared with compound IIb (Table 2). Among compounds I, the oxadiazine derivatives did not show any significant change in antitumor activity as compared with the thia derivatives. In relation to NCI-H460 and MCF7 tumors, compounds If, g had rather greater activity than compound Ib. More detailed testing of oxadiazine derivatives If, g using nine groups of tumor cells showed that the antitumor activity of these tricyclic fluoroquinolones was significantly less than that of compounds II.

The data presented in Table 3 show that thiadiazinoquinolines containing a cyclohexylamine fragment at position 3 (compounds Ic-e) were shown by the Samsung data to show significant selectivity in relation to SK-OV-3 cells (the percentage death of tumor cells ranged from 55% to 90%).

In vivo experiments using hollow fibers were performed using a selection of compounds at the next stage of the study (using a xenographic model). These experiments showed that the pentacyclic quinolone IIb produced complete cell death (Table 4).

Thus, pentacyclic fluoroquinolones II have the greatest potential in the search for antitumor agents.

This study was supported by State Contract GK-02.740.11.0260 and Scientific Schools grant No. NSh-65261.2010.3.

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