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SYNTHESIS AND CYTOTOXIC ACTIVITY OF FLUORINE-CONTAINING 6,7-DIHYDROINDAZOLONE AND 6,7-DIHYDROBENZISOXAZOLONE DERIVATIVES

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The oximes of fluorine-containing 6,7-dihydroindazole-4,5-diones and 6,7-dihydrobenzisoxazole-4-5-diones, 5-[3-fluorobenzoyloxy)imino)]-6,6-dimethyl-3-(3-fluorophenyl)-6,7-dihydrobenz[*d*]]isoxazol-4(5*H*)-one, and 5,5-dimethyl-7-phenyl-9-(4-fluorophenyl)-3,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-*f*]quinoxaline were synthesized. The cytotoxic activity of these compounds against cell lines MCF-7 (human breast carcinoma) and HepG2 (human hepatocellular carcinoma) was determined. The fluorine-containing 6,7-dihydroindazolone derivatives synthesized here included substances with marked antiproliferative activity, the mechanism of which may be linked with induction of apoptosis due to impairment of the regular cell cycle, i.e., delayed of tumor cells in the G2/M phase.

Keywords: 3-[fluoroalkyl(aryl)]-6,7-dihydroindazolone derivatives; 3-[fluoroalkyl(aryl)]-6,7-dihydrobenzisoxazolone derivatives, synthesis, cytotoxic activity.

Many heterocyclic compounds containing pyrazole and isoxazole rings (both alone and condensed with other mono- or polycyclic systems) are used as the base structures for the design of numerous pharmacological agents [1, 2]. Derivatives of indazole [3] and benzisoxazole [4] have a wide spectrum of biological activity, including antitumor, anti-HIV, anti-inflammatory, analgesic, antipsychotic, and other activities. The main targets of the antitumor actions of indazole and benzisoxazole derivatives are protein kinases, as well as heat shock protein HSP90, which is involved in regulating the cell cycle; this can lead to the induction of apoptosis [5, 6]. An effective strategy for developing novel therapeutic agents consists of selective introduction of fluorine atoms and fluoroalkyl groups into biologically active molecules. This is illustrated by the continuing increase in the number of fluorine-containing drugs already approved for use and in clinical trials [7]. Methods for synthesizing fluorine-containing heterocyclic structures as potential drugs are currently under intense development [8, 9].

The aim of the present work was to synthesize a group of novel 6,7-dihydroindazolone and 6,7-dihydrobenzisoxazolone derivatives, namely the oximes of fluorine-containing 6,7-dihydroindazole-4,5-diones and 6,7-dihydrobenzisoxazole-4-5-diones, as well as 5,5-dimethyl-7-phenyl-9-(4-fluorophenyl)-3,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-*f*]-quinoxaline and 5-[3-fluorobenzoyloxy)imino)]-6,6-dimethyl-3-(3-fluorophenyl)-6,7-dihydrobenz[*d*]]isoxazol-4(5*H*)-one, and to study their cytotoxic activities against cell lines MCF-7 (human breast carcinoma) and HepG2 (human hepatocellular carcinoma).

The oximes of fluorine-containing 6,7-dihydroindazole-4,5-diones and 6,7-dihydrobenzisoxazole-4,5-diones were prepared using a synthesis approach based on 6,7-dihydroindazolones and 6,7-dihydrobenzisoxazolones including oxidation of these heterocycles on exposure to selenium dioxide followed by oximation of the resulting dioxo derivatives with hydroxylamine hydrochloride. Thus, boiling of indazo-

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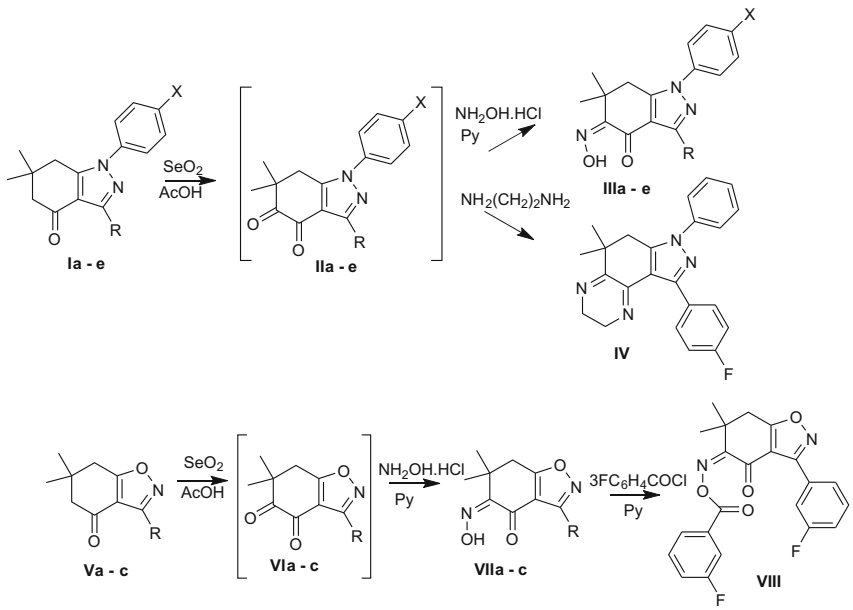

I – **III**: R = CF_3, X = H (**a**); R = C_2F_5, X = H (**b**); R = 3F-C_6H_4, X = H (**c**); R = 4F-C_6H_4, X = H (**d**); R = 4F-C_6H_4, X = F (**e**); V-VII: R = CF_3 (**a**); 3F-C_6H_4 (**b**); 4F-C_6H_4 (**c**).

lones **Ia – e** in glacial acetic acid solution with a threefold excess of selenium dioxide for 3 h and processing yielded 5,6-dihydroindazole-4,5-diones **IIa – e**, which without additional purification were treated with equimolar quantities of hydroxylamine hydrochloride in pyridine for 20 h to produce oximes **IIIa – e** with yields of 43 – 69%. Condensation of 6,7-dihydroindazole-4,5-dione **IIc** with an equivalent quantity of ethylenediamine in ethanol with boiling for 3 h produced 3,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-*f*]quinoxaline (**IV**) with a yield of 58%. The oximes of benzisoxazole-4,5-diones **VIIa – c** were synthesized in conditions analogous to those used for preparation of oximes **IIIa – e**, though the duration of the oxidation reaction for benzisoxazolones **Va – c** had to be increased to 8 h and the subsequent oximation of the resulting dioxo derivatives **Va – c** had to be increased to 72 h. The oximation reaction was run with a four-fold excess of hydroxylamine hydrochloride. The yields of oximes **VIIa – c** were 59 – 62%. Acylation of the oxime of 6,7-dihydrobenzisoxazolone **VIIb** with 3-fluorobenzoic acid chloranhydride in chloroform in the presence of pyridine for 20 h produced 5-[(3-fluorobenzoyloxy)imino]-6,6-dimethyl-3-(3-fluorophenyl)-6,7-dihydrobenz[*d*]isoxazol-4(5*H*)-one (**VIII**) with a yield of 80%.

The structures of all compounds synthesized here were confirmed by ^1H, ^{13}C, and ^{19}F NMR spectroscopy and elemental analysis. Along with signals from methyl and ethylene group protons and aromatic protons, the ^1H NMR spectra of the oximes of **IIIa – e** and **VIIa – c** contained signals from the hydroxyl proton as a singlet at δ 14.81 – 15.14 ppm and δ 14.13 – 14.45 ppm respectively, indicating the presence of strong intramolecular bonds. The ^{13}C NMR spectra of oximes **IIIa – e** and **VIIa – c** contained signals from the C^4 carbonyl group carbon atom in the range δ 178.7 – 182.5 ppm and the C^5 carbon atom bound to the hydroxyimine group at δ 152.3 – 153.8 ppm. Analysis of two-dimensional NMR spectra for oxime IIIa showed that oximation occurred at the C^5 carbonyl group, as is also the case for their nonfluorinated analogs [10, 11]. The position of C^5 was determined from the interaction between protons at C^7 and the methyl protons with this nucleus in the HMBC spectrum.

Oximes **IIIa – e** and **VIIa – c** can exist in two configurations – (*Z*) and (*E*). Structures were confirmed by x-ray anal-

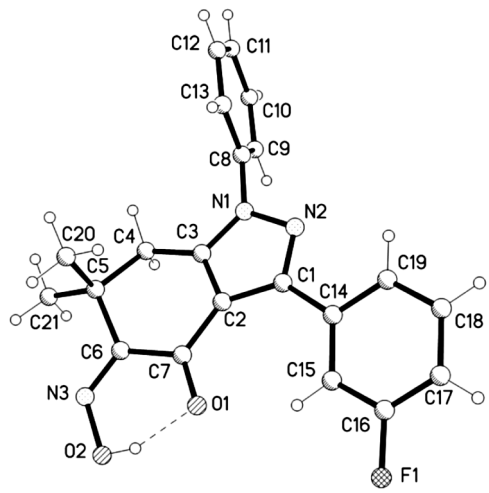

Fig. 1. Molecular structure of oxime **IIIc**.

ysis (XRA) of monocrystals of compound I**IIc**. Figure 1 shows the XRA of compound I**IIc**, confirming that this compound is in the *Z* configuration.

EXPERIMENTAL CHEMICAL SECTION

^{1}H, ^{13}C, and ^{19}F NMR spectra were obtained for solutions in $CDCl_3$ using a Bruker Biospin Avance 500 spectrometer with working frequencies of 500.13, 470.59, and 125.77 MHz for ^{1}H, ^{19}F, and ^{13}C nuclei respectively. The internal standard for ^{1}H NMR was the residual solvent signal δ_H 7.26 ppm ($CDCl_3$) and the internal standard for ^{13}C NMR was the residual solvent signal δ_C 77.16 ppm ($CDCl_3$), and the external standard was the signal from α,α,α-trifluorotoluene δ 63 ppm (^{19}F). Correlational spectra (HSQC, COSY, HMBC, NOESY) were recorded and processed using standard software from Bruker Biospin. Crystals of oxime **IIIc** were rhombic, spatial group Pba, a = 12.090(3), b = 10.522(3), c = 28.570(7) Å, V = 3634.6(16) Å^3. XRA of a **IIIc** monocrystal was run on a Bruker Smart Apex II diffractometer (λMoKα-radiation, θ_{max} 25.05°). A total of 16,180 reflections were acquired, of which 3219 were independent; structures were deciphered by a direct method and refined by the least squares method and full-matrix anisotropic approximation run in SHELXTL [12]. Final values for divergence factors R1(F) 0.0861, wR2(F2) 0.1758, GOF 1.055 were from reflections with $I > 2\sigma(I)$. Melting temperatures were measured on a Boetius apparatus. Elemental analysis was run on a EuroVector EA3000 CHNS-O

TABLE 1. Spectral Characteristics of compounds **IIIa – e**, **IV**, **VIIa – c** and **VIII**

Compound	^{1}H NMR spectrum, δ, ppm	^{13}C NMR spectrum, δ, ppm	^{19}F NMR spectrum, δ, ppm
IIIa	1.36 (s, 6H, 2CH_3), 3.04 (s, 2H, CH_2), 7.46 – 7.51 (m, 2H, H_{arom}), 7.53 – 7.62 (m, 3H, H_{arom}), 14.81 (s, 1H, OH)	27.5, 36.9, 40.4, 115.8, 120.1 (q, J_{C-F} 269 Hz), 124.4, 130.0, 137.1, 142.1 (q, J_{C-F} 40 Hz), 150.4, 153.0, 178.7	–63.31 (3F)
IIIb	1.35 (s, 6H, 2CH_3), 3.05 (s, 2H, CH_2), 7.47 – 7.52 (m, 2H, H_{arom}), 7.53 – 7.62 (m, 3H, H_{arom}), 14.85 (s, 1H, OH)	27.4, 36.9, 40.2, 110.3 (t.q., J_{C-F} 253, 40 Hz), 117.0, 118.8 (qt, J_{C-F} 287, 37 Hz), 124.4, 130.0, 137.1, 141.2 (t, J_{C-F} 31 Hz), 150.4, 153.0, 179.0	–82.84 (3F), –111.39 (2F)
IIIc	1.37 (s, 6H, 2CH_3), 3.05 (s, 2H, CH_2), 7.11 – 7.18 (m, 1H, H_{arom}), 7.39 – 7.48 (m, 1H, H_{arom}), 7.49–7.62 (m, 5H, H_{arom}), 7.85 – 7.92 (m, 1H, H_{arom}), 7.92 – 7.98 (m, 1H, H_{arom}), 15.14 (s, 1H, OH)	27.5, 37.1, 40.1, 115.9, 116.1, 116.6 (d, J_{C-F} 21 Hz), 124.4, 124.8, 129.4, 129.8, 129.9, 133.1 (d, J_{C-F} 9 Hz), 137.8, 150.1, 152.4, 153.8, 162.8 (d, J_{C-F} 245 Hz), 180.5	–113.00 (1F)
IIId	1.37 (s, 6H, 2CH_3), 3.04 (s, 2H, CH_2), 7.10 – 7.19 (m, 2H, H_{arom}), 7.47 – 7.61 (m, 5H, H_{arom}), 8.09 – 8.16 (m, 1H, H_{arom}), 15.13 (s, 1H, OH)	27.5, 37.1, 40.2, 115.4 (d, J_{C-F} 22 Hz), 115.8, 124.4, 127.2, 129.3, 129.8, 131.1 (d, J_{C-F} 9 Hz), 137.8, 150.0, 152.7, 153.8, 163.8 (d, J_{C-F} 250 Hz), 180.6	–111.19 (1F)
IIIe	1.37 (s, 6H, 2CH_3), 3.00 (s, 2H, CH_2), 7.12 – 7.18 (m, 2H, H_{arom}), 7.23 – 7.30 (m, 2H, H_{arom}), 7.48–7.55 (m, 2H, H_{arom}), 8.07 – 8.14 (m, 2H, H_{arom}), 15.10 (s, 1H, OH)	27.6, 37.0, 40.2, 115.5 (d, J_{C-F} 22 Hz), 115.8, 116.9 (d, J_{C-F} 23 Hz), 126.4 (d, J_{C-F} 9 Hz), 127.0, 131.1 (d, J_{C-F} 9 Hz), 133.9, 150.1, 152.8, 153.7, 162.7 (d, J_{C-F} 250 Hz), 163.8 (d, J_{C-F} 250 Hz), 180.5	–110.77 (1F), –111.02 (1F)
IV	1.26 (s, 6H, 2CH_3), 2.98 (s, 2H, CH_2), 3.52 (s, 4H, 2CH_2), 7.10 (t, 2H, H_{arom}, J 8.6 Hz), 7.40 – 7.48 (m, 1H, H_{arom}), 7.48 – 7.60 (m, 4H, H_{arom}), 8.18 (dd, J 8.6 Hz, 5.5, 2H, H_{arom})	26.4, 37.1, 40.0, 44.5, 45.3, 114.4, 114.9 (d, J_{C-F} 21 Hz), 124.2, 128.3, 128.8, 129.6, 131.5 (d, J_{C-F} 8 Hz), 138.7, 143.7, 150.2, 150.5, 163.2 (d, J_{C-F} 248 Hz), 165.9	-113.22 (1F)
VIIa	1.43 (s, 6H, 2CH_3), 3.19 (s, 2H, CH_2), 14.13 (s, 1H, OH)	27.7, 37.0, 40.1, 112.8, 118.8 (q, J_{C-F} 272 Hz), 152.0 (q, J_{C-F} 41 Hz), 152.3, 177.6, 182.5	-63.53 (3F)
VIIb	1.43 (s, 6H, 2CH_3), 3.16 (s, 2H, CH_2), 7.21 – 7.27 (m, 1H, H_{arom}), 7.48 (td, J_{C-F} 8.0 Hz, 5.8, 1H, H_{arom}), 7.80 (dt, J_{C-F} 9.7 Hz, 2.1, 1H, H_{arom}), 7.85 (dt, J_{C-F} 7.7 Hz, 1.3, 1H, H_{arom}), 14.45 (s, 1H, OH)	27.7, 37.2, 40.0, 113.7, 116.5 (d, J_{C-F} 24.0 Hz), 118.3 (d, J_{C-F} 21.0 Hz), 125.1, 128.4 (d, J_{C-F} 8 Hz), 130.4 (d, J_{C-F} 8 Hz), 153.1, 159.8, 162.7 (d, J_{C-F} 247 Hz), 180.1, 182.2	-111.77 (1F)
VIIc	1.43 (s, 6H, 2CH_3), 3.15 (s, 2H, CH_2), 7.16 – 7.22 (m, 2H, H_{arom}), 8.04 – 8.09 (m, 2H, H_{arom}), 14.45 (s, 1H, OH)	27.7, 37.3, 39.9, 113.7, 116.0 (d, J_{C-F} 22 Hz), 122.6, 131.6 (d, J_{C-F} 9 Hz), 153.1, 159.9, 164.7 (d, J_{C-F} 252 Hz), 180.2, 182.1	-108.28 (1F)
VIII	1.55 (s, 6H, 2CH_3), 3.26 (s, 2H, CH_2), 7.20 – 7.28 (m, 1H, H_{arom}), 7.30 (td, J 8.3 Hz, 2.7, 1H, H_{arom}), 7.41 – 7.57 (m, 2H, H_{arom}), 7.76 (dt, J 9.3 Hz, 2.0, 1H, H_{arom}), 7.83 – 7.91 (m, 2H, H_{arom}), 7.91 – 8.00 (m, 1H, H_{arom})	25.7, 38.6, 41.4, 115.3, 116.2 (d, J_{C-F} 24 Hz), 117.0 (d, J_{C-F} 24 Hz), 118.3 (d, J_{C-F} 21 Hz), 121.0 (d, J_{C-F} 21 Hz), 124.9 (d, J_{C-F} 3 Hz), 125.9 (d, J_{C-F} 3 Hz), 128.6 (d, J_{C-F} 9 Hz), 130.4 (d, J_{C-F} 9 Hz), 130.5 (d, J_{C-F} 2 Hz), 130.5 (d, J_{C-F} 3 Hz), 159.4, 162.4, 162.7 (d, J_{C-F} 248 Hz), 162.8 (d, J_{C-F} 247 Hz), 164.8, 176.2, 180.6	-111.71 - (-111.85) (m), -111.48 - (-111.65) (m)

analyzer. Elemental analysis data were consistent with calculated values. Column chromatography (CC) was run on silica gel eluted with a mixture of ethyl acetate and petroleum ether. 6,7-Dihyroindazolones **Ia – e** [13, 14] and 6,7-dihydrobenzisoxazolones **Va – c** [15, 16] were prepared by standard methods.

Oximes of 3-[fluoroalkyl(aryl)]-6,7-dihydroindazole-4,5-diones (IIIa – e). Selenium dioxide (0.27 g, 2.4 mmol) was added to a solution of 0.8 mmol of 6,7-dihydroindazolone **Ia – e** in 25 ml of glacial acetic acid and the solution was boiled for 3 h. Acetic acid was removed on a rotary evaporator. Chloroform (40 ml) was added to the residue and filtered and the chloroform was removed in a rotary evaporator. The residue was dissolved in 4 ml of pyridine and 0.06 g (0.8 mmol) of hydroxylamine hydrochloride was added with mixing for 20 h at room temperature. The reaction mix was poured into a mixture of 60 ml of 18% HCl and 20 g of ice and extracted with chloroform (5 × 20 ml). The combined organic layers were dried over Na_2SO_4. Solvent was removed on a rotary evaporator and CC was used to extract oximes **IIIa – e** as colorless crystals.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-1-phenyl-3-trifluoromethyl-1,5,6,7-tetrahydro-4*H*-indazol-4-one (IIIa). The yield was 55%. T_m was 107 – 110°C. $C_{16}H_{14}F_3N_3O_2$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-1-phenyl-3-perfluoroethyl-1,5,6,7-tetrahydro-4*H*-indazol-4-one (IIIb). The yield was 69%. T_m was 111 – 114°C. $C_{17}H_{14}F_5N_3O_2$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-1-phenyl-3-(3-fluorophenyl)-1,5,6,7-tetrahydro-4*H*-indazol-4-one (IIIc). The yield was 43%. T_m was 145 – 148°C. $C_{21}H_{18}FN_3O_2$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-1-phenyl-3-(4-fluorophenyl)-1,5,6,7-tetrahydro-4*H*-indazol-4-one (IIId). The yield was 48%. T_m was 159 – 162°C. $C_{21}H_{18}FN_3O_2$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-1,3-bis(4-fluorophenyl)-1,5,6,7-tetrahydro-4*H*-indazol-4-one (IIIe). The yield was 66%. T_m was 147 – 149°C. $C_{21}H_{17}F_2N_3O_2$.

5,5-Dimethyl-7-phenyl-9-(4-fluorophenyl)-3,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-*f*]**quinoxaline (IV)**. Selenium dioxide (0.17 g, 1.5 mmol) was added to a solution of 0.17 g (0.5 mmol) of indazolone Id in 15 ml of glacial acetic acid and the mixture was boiled for 3 h. Acetic acid was removed in a rotary evaporator. Chloroform (40 ml) was added to the residue and filtered; the chloroform was evaporated in a rotary evaporator. The residue was dissolved in 15 ml of ethanol and 0.03 ml (0.5 mmol) of ethylenediamine was added and the mixture was boiled for 3 h. Solvent was removed in a rotary evaporator and CC was used to extract 0.104 g (58%) of compound **IV** from the residue in the form of colorless crystals. T_m was 158 – 161°C. $C_{23}H_{21}FN_4$.

Oximes of 3-[fluoroalkyl(aryl)]-6,7-dihydrobenzisoxazole-4,5-diones (VIIa – c). Selenium dioxide (0.83 g, 7.5 mmol) was added to a solution of 2.5 mmol of isoxazolone **Va – c** in 45 ml of glacial acetic acid and boiled for 8 h. Acetic acid was removed in a rotary evaporator. Chloroform (80 ml) was added to the residue and filtered; the chloroform was removed in a rotary evaporator. The residue was dissolved in 10 ml pyridine and 0.70 g (10.0 mmol) of hydroxylamine hydrochloride was added and mixed at room temperature for 72 h. The reaction mix was poured into a mixture of 120 ml of 18% HCl and 40 g of ice and extracted with chloroform (5 × 30 ml). The combined organic layers were dried over Na_2SO_4. Solvent was removed in a rotary evaporator and CC was used to extract oximes **VIIa – c** as colorless crystals.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-3-(trifluoromethyl)-6,7-dihydrobenz[*d*]isoxazol-4(5*Í*)-one (VIIa). The yield was 61%. T_m was 158 – 161°C. $C_{10}H_9F_3N_2O_3$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-3-(3-fluorophenyl)-6,7-dihydrobenz[*d*]isoxazol-4(5*Í*)-one (VIIb). The yield was 62%. T_m was 138 – 140°C. $C_{15}H_{13}FN_2O_3$.

(*Z*)-5-(Hydroxyimino)-6,6-dimethyl-3-(4-fluorophenyl)-6,7-dihydrobenzî[*d*]isoxazol-4(5*Í*)-one (VIIc). The yield was 59%. T_m was 145 – 148°C. $C_{15}H_{13}FN_2O_3$.

(*Z*)-6,6-Dimethyl-5-[(3-fluorobenzoyloxy)imino]-3-(3-fluorophenyl)-6,7-dihydrobenz[*d*]isoxazol-4(5*H*)-one (VIII). Pyridine (0.1 ml) was added to a solution of 0.05 g (0.17 mmol) of oxime **VIIb** in 15 ml of chloroform, followed by 0.036 g (0.26 mmol) of 3-fluorobenzoic acid chloranhydride. The reaction was mixed for 20 h, washed with 3% HCl (2 × 5 ml), and dried over Na_2SO_4. Compound **VIII** was extracted from the residue by CC as colorless crystals. The yield was 0.057 g (80%). T_m was 152 – 155°C. $C_{22}H_{16}F_2N_2O_4$.

EXPERIMENTAL BIOLOGICAL SECTION

Studies of the cytotoxicity of 6,7-dihydroindazolone derivatives **IIIa – e** and 6,7,-dihydrobenzisoxazolone derivatives **VIIa – c**, **VIII** were performed using the MTT test [17]. MCF-7 (human breast carcinoma) and HepG2 (human hepatocellular carcinoma) cells obtained from the Russian Collection of Cell Cultures (Institute of Cytology, Russian Academy of Sciences, St. Petersburg) were cultured in DMEM and MEM nutrient media respectively. Media were supplemented with 10% fetal bovine serum and a mixture of

TABLE 2. Effects of Compounds **IIIb – e** on Cell Cycle Phases of HepG2 Tumor Cells

Compound	Cell cycle phase, %			Apoptosis, %
	G1	S	G2/M	
Control	63.07 ± 4.56	13.30 ± 3.47	23.88 ± 3.40	1.56 ± 0.38
Cisplatin	36.55 ± 3.25	2.54 ± 0.72	60.91 ± 3.97	32.63 ± 2.26
IIIb	37.44 ± 6.99	12.57 ± 3.52	49.99 ± 3.47	19.50 ± 3.25
IIIc	38.01 ± 3.95	6.63 ± 4.57	55.36 ± 0.62	13.97 ± 4.19
IIId	31.00 ± 0.63	21.25 ± 3.99	46.92 ± 3.90	19.59 ± 3.14
IIIe	40.68 ± 2.39	19.65 ± 2.35	39.69 ± 4.74	15.44 ± 4.23

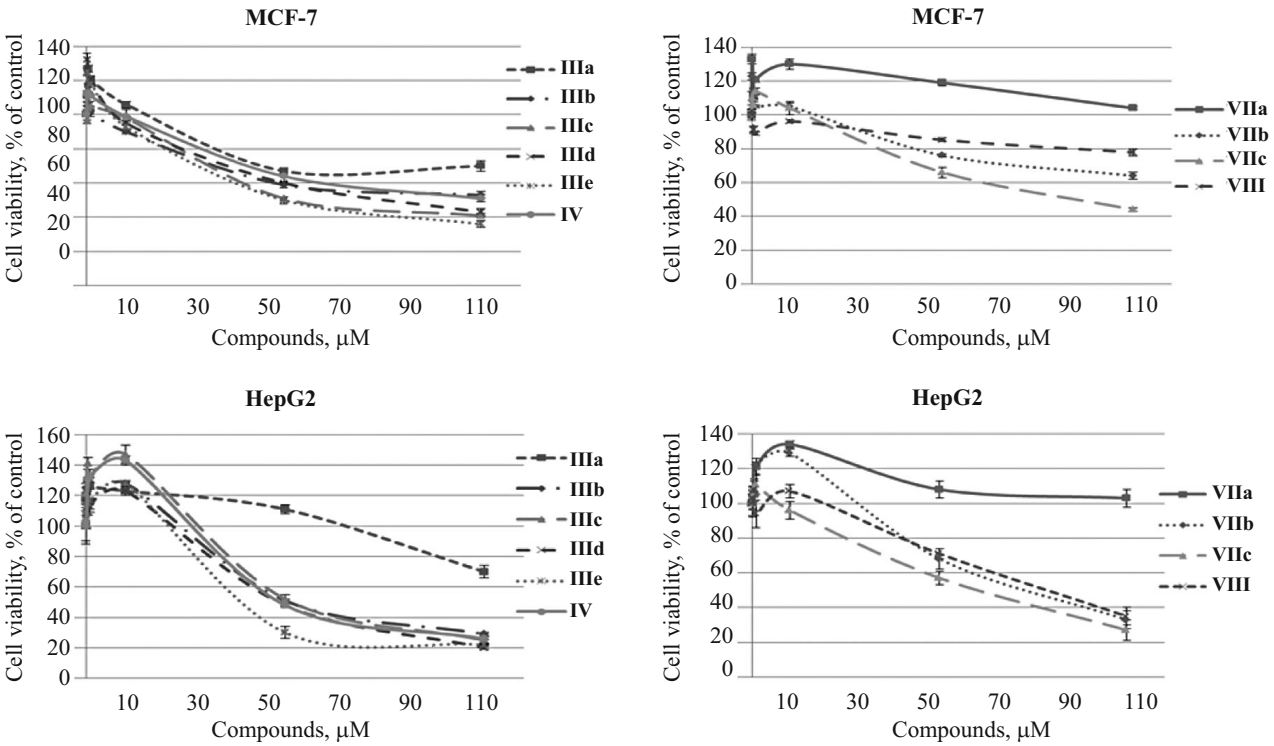

Fig. 2. Viability of MCF-7 and HepG2 cells after treatment with solutions of compounds **IIIa – e**, **IV**, **VIIa – c**, and **VIII**.

penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (25 μg/ml) and incubation was at 37°C in a humid atmosphere containing 5% CO_2. Cells of both lines were seeded into 96-well plates at a concentration of 7000 cells/well and were incubated for 24 h. Test substances were added to concentrations of 0.1, 0.5, 1, 10, 50, and 100 μM. The concentration of stock solutions of test compounds was 20 mM. Solutions were added to the final concentrations in incubation medium. Controls were supplemented with 0.5% solvent. After 72 h, each well was supplemented with 20 μl MTT (5 mg/ml). After 3 h exposure at 37°C and 5% CO_2, granules of reduced product (formazan) were dissolved in 200 μl of DMSO and the optical density of the solution was measured at 570 nm on an AIF-M/340 plate reader. Percent viability = OD experimental wells/OD control wells ×100%, where OD is optical density.

Drug concentrations inhibiting cell viability by 50% (IC_{50}) were determined graphically using the dose curve in Excel. All experiments were run in three repeats. Statistical significance was at $p < 0.05$. Analysis of the effects of synthetic compounds on the distribution of HepG2 cells in the cell cycle and the types of cell death (apoptosis or necrosis) induced was performed using a Beckman Coulter FC500 flow cytometer in the FL-3 channel. Levels of apoptosis were determined using the "subG1" peak. Analyses were run using Kaluza software (Beckman Coulter).

HepG2 cells were seeded at a rate of 300,000 cells/Petri dish (6 cm^2). After 24 h, culture medium was replaced with medium containing test compounds at the IC_{50} determined in the MTT test. After 48 h, cells were removed from plates and fixed with cold 70% ethanol at -20°C. Cells were then stained with propidium iodide (50 μg/ml) + RNase A (1 mg/ml) in phosphate buffer for 30 min in the dark at room temperature. The reference agent was cisplatin, with IC_{50} 17 μM. Experiments were performed in three repeats. Statistical significance was $p < 0.05$.

Results from the MTT test are shown in Fig. 2. Among the study compounds, only benzisoxazolone oxime VIIa had no cytotoxic effect in relation to both lines. The activity of the other compounds was dose-dependent: low concentrations (0.1 – 1 μM) stimulated tumor cell growth, while high concentrations (>10 μM) inhibited growth. Indazolone derivatives **IIIb – e**, **IV** decreased tumor cell growth in both lines at a concentration of 50 μM by ?50%, while a concentration of 100 μM decreased the growth of HepG2 cells by 80% and that of MCF-7 by 64%. Isoxazolone derivatives **VIIb**, c and **VIII** at 50 μM inhibited the growth of cells of both lines less effectively: by only 30 – 40%. Overall, human liver carcinoma cells HepG2 were more sensitive to the actions of the study compounds.

The effects of indazolone derivatives **IIIb – e** most active against HepG2 cells on the distribution of HepG2 cells by cell cycle phase and the type of cell death (apoptosis of the necrosis) induced by compounds were studied (Table 2; Figs. 3 and 4). The IC_{50} values of these compounds were in the range 40 – 60 μM. Compounds **IIIb – e** terminated the

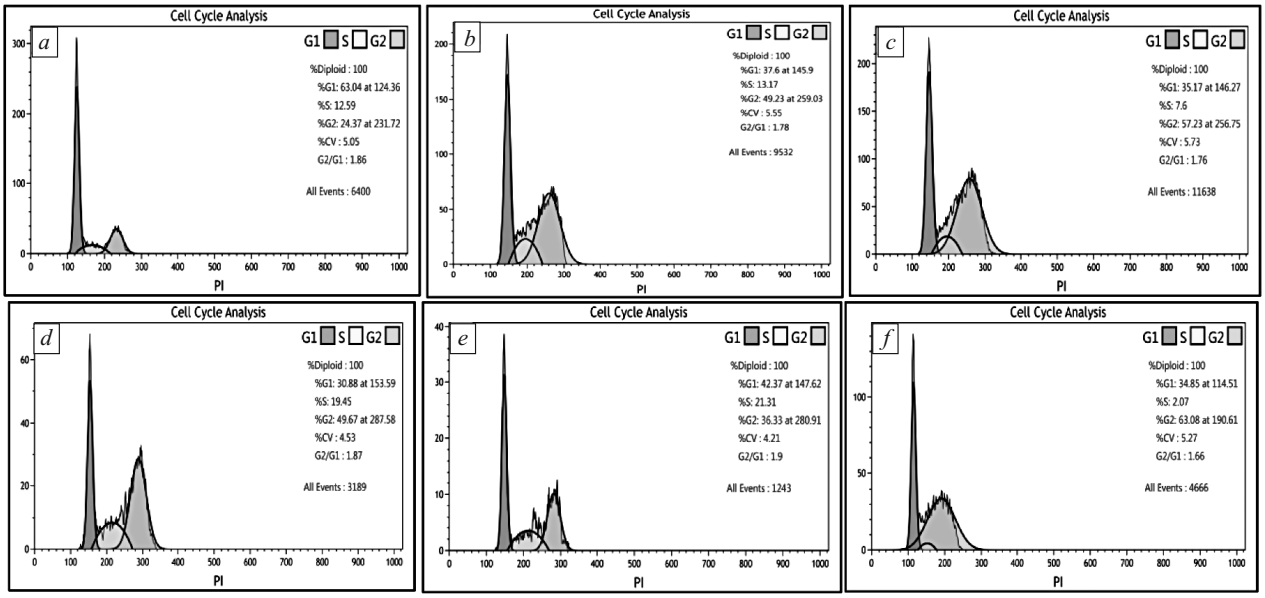

Fig. 3. Distributions of HepG2 cells in the cell cycle after exposure to test compounds: *a*) controls; *b*) **IIIb**; *c*) **IIIc**; *d*) **IIId**; *e*) **IIIe**; *f*) cisplatin.

cell cycle in the G2/M phase, increasing the proportion of cells in this phase twofold from the level in controls and decreasing the proportion in G1. They had virtually no effect on the S phase (except compound **IIIc**). The result of termination of the cell cycle was an increase in the level of apoptosis from 2% in controls to 13 – 19% with study compounds. Cisplatin increased the level of apoptosis to 33%. The mechanism of the cytotoxic action of study compounds in relation to tumor cells appears to be associated with induction of cell death by the mechanism of apoptosis due to impairment to the regular cell cycle, i.e., delay of tumor cells in the G2/M phase.

Thus, among the fluorine-containing 6,7-dihydroindazolone derivatives synthesized here, substances with marked antiproliferative activity were found, which is evidence of the value of continuing studies of this series of compounds.

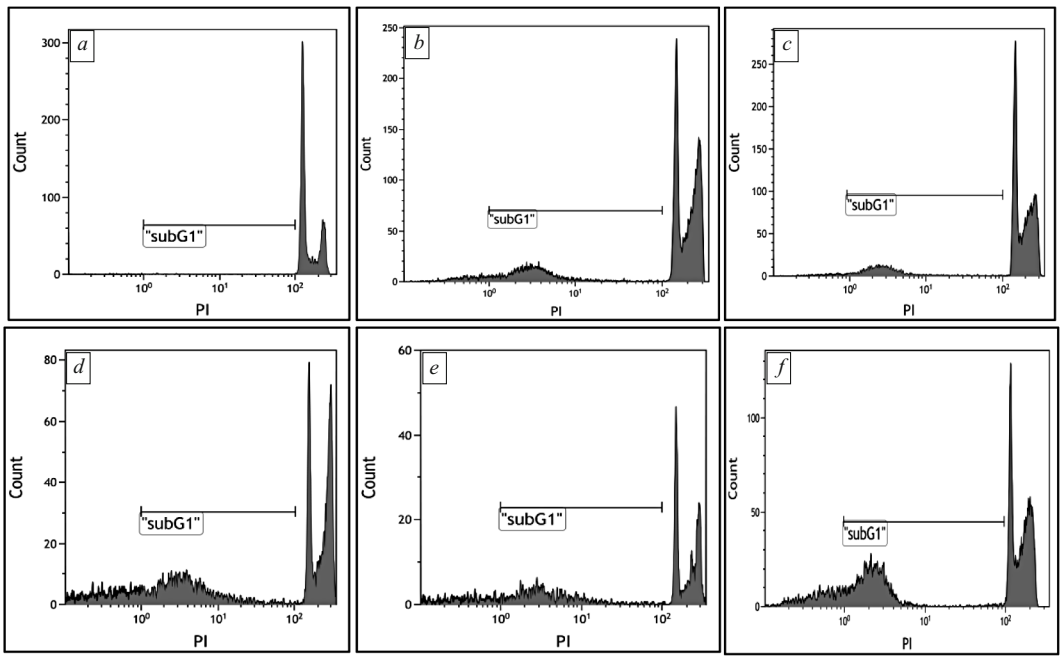

Fig. 4. Level of apoptosis of HepG2 cells after exposure to test compounds: *a*) controls; *b*) **IIIb**; *c*) **IIIc**; *d*) **IIId**; *e*) **IIIe**; *f*) cisplatin.

REFERENCES

1. D. D. Gaikwad, A. D. Chapolikar, C. G. Devkate, et al., *Eur. J. Med. Chem.*, **90**, 707 – 731 (2015).
2. J. Zhu, J. Mo, H-Z. Lin, et al., *Bioorg. Med. Chem.*, **26**(12), 3065 – 3075 (2018).
3. S. Zhang, C. Liang, and W. Zhang, *Molecules*, **23**, 2783 (2018); DOI: 10.3390 / molecules23112783.
4. K. P. Rakesh, C. S. Shantharam, M. B. Sridhara, et al., *Med. Chem. Commun.*, **8**(11), 2023 – 2039 (2017).
5. I. Denya, S. F. Malan, and J. Joubert, *Expert Opin. Ther. Pat.*, **28**(6), 441 – 453 (2018).
6. L. Li, L. Wang, Q-D. You, et al., *J. Med. Chem.*, **63**(5), 1778 – 1805 (2020).
7. Yu Zhou, J. Wang, Z. Gu, et al., *Chem. Rev.*, **116**(2), 422 – 518 (2016).
8. V. G. Isakova, T. S. Khlebnikova, and F. A. Lakhvich, *Usp. Khim.*, **79**(10), 929 – 960 (2010); V. G. Isakova, T. S. Khlebnikova, and F. A. Lakhvich, *Rus. Chem. Rev.*, **79**(10), 849 – 879 (2010).
9. L. V. Politanskaya, G. A. Selivanova, E. V. Panteleeva, et al., *Usp. Khim.*, **88**(5), 425 – 569 (2019); L. V. Politanskaya, G. A. Selivanova, E. V. Panteleeva, et al., *Rus. Chem. Rev.*, **88**(5), 425 – 569 (2019).
10. D. R. Zitsane and A. Ya. Strakov, *Izv. Akad. Nauk. Latv. SSR Ser. Khim.*, No. 6, 742 – 744 (1975).
11. A. Ya. Strakov and M. B. Andaburskaya, *Izv. Akad. Nauk. Latv SSR Ser. Khim.*, No. 1, 108 – 110 (1975).
12. G. Sheldrick, *Acta Cryst.*, **A64**(1), 112 – 122 (2008).
13. T. S. Khlebnikova, V. G. Isakova, A. V. Baranovskii, et al., *Zh. Obshch. Jhim.*, **78**(10), 1718 – 1727 (2008); T. S. Khlebnikova, V. G. Isakova, A. V. Baranovskii, et al., *Rus. J. Gen. Chem.*, **78**(10), 1954 – 1963 (2008).
14. T. S. Khlebnikova, V. G. Isakova, and F. A. Lakhvich, *Izv. Nats. Akad. Nauk Belarusi Ser. Khim. Nauk*, No. 3, 79 – 83 (2009).
15. T. S. Khlebnikova, Yu. A. Piven', V. G. Isakova, et al., *Khim. Geterotsikl. Soedin.*, **53**(11), 1254 – 1260 (2017); T. S. Khlebnicova, Yu. A. Piven', V. G. Isakova, et al., *Chem. Heterocycl. Comp.*, **53**(11), 1254 – 1260 (2017).
16. T. S. Khlebnicova, Yu. A. Piven', V. G. Isakova, et al., *J. Heterocycl. Chem.*, **55**(7), 1791 – 1797 (2018).
17. J. van Meerloo, G. J. Kaspers, and J. Cloos, *Methods Mol. Biol.*, **731**, 237 – 245 (2011).