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SYNTHESIS, ANTICANCER AND FGFR$_1$ INHIBITORY ACTIVITY OF ISOINDOLO[2,1-*a*][1,2,4]TRIAZINO[2,3-*c*]QUINAZOLINE DERIVATIVES

Presented manuscript describes the synthesis, antitumor and FGFR$_1$ inhibitory activity of novel isoindolo[2,1-a][1,2,4]triazino[2,3-c]quinazolines. It was shown that mentioned above substances may be prepared by interaction of 3-(2-amino-3-R$_2$-5-R$_3$-phenyl)-6-R$_1$-1,2,4-triazin-5(2H)-ones with 2-formylbenzoic and 6-formyl-2,3-dimethoxybenzoic (opianic) acids in acetic acid. It was shown that proper 2-(2-oxo-3-R-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)benzoic acids (or corresponded dimethoxysubstituted analogues) may be considered as intermediates of the reaction. Spectral properties of synthesized compounds were studied, it was shown that protons in position 8 were observed at low field as result of the presence of hydrogen bond between hydrogen at position 8 and oxygen at position 10. The anticancer assay data allowed to identify synthesized compounds as promising antitumor agents. The FGFR$_1$ inhibitory activity of synthesized compounds was detected and docking study aimed to the evaluation of mentioned action was conducted.

KEY WORDS: **isoindoles, triazines, quinazolines, anticancer activity, FGFR$_1$ inhibitory activity, docking.**

INTRODUCTION. Recently, isoindole derivatives have been considered as promising compounds for the development of novel anticancer agents. Significant interest to this class of the heterocyclic compounds is caused by a series of publications which reported the natural and synthetic compounds able to decrease malignant cell growth or inhibit activities of key enzymes. For instance, it was shown that aristolactam BII has moderate antitumor activity in human cell lines in vitro [1]. Significant anticancer activity toward HCT-116 cell line was also established for (-)-chloirzidine A – compound which was obtained as product of marine Streptomyces strain cultivation [2]. Promising results were obtained for some synthetic compounds with isoindole moiety. For example, anticancer agents were identified among compounds containing condensed isoindole and benzodiazine fragments. The antitumor activity was revealed for isoindolo[2,1-a]quinoxalin-6(5H)-ones (i) and benzo[5,6]isoindolo[1,2-a]phthalazine-9,14-diones (ii) [3, 4], and moreover, well-known antitumor agent luteolin A [5] contains fragment, which may be considered as *aza*-analog of isoindolo[1,2-b]quinazoline system (Figure 1).

Thus, combination of isoindole and benzodiazine fragments might become a rational approach for constructing of novel compounds with anticancer activity. Hence, considering promising antitumor activity of previously described compounds [6–8] we decided to combine [1,2,4]triazino[2,3-c]quinazoline fragment and isoindole moiety in one molecule with the following study of anticancer and enzyme inhibitory activity of obtained compounds.

METHODS OF RESEARCH.

Experimental Section

Synthetic chemistry

Melting points were determined in open capillary tubes and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL Cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3 % of the theoretical values. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Varian-Mercury 400

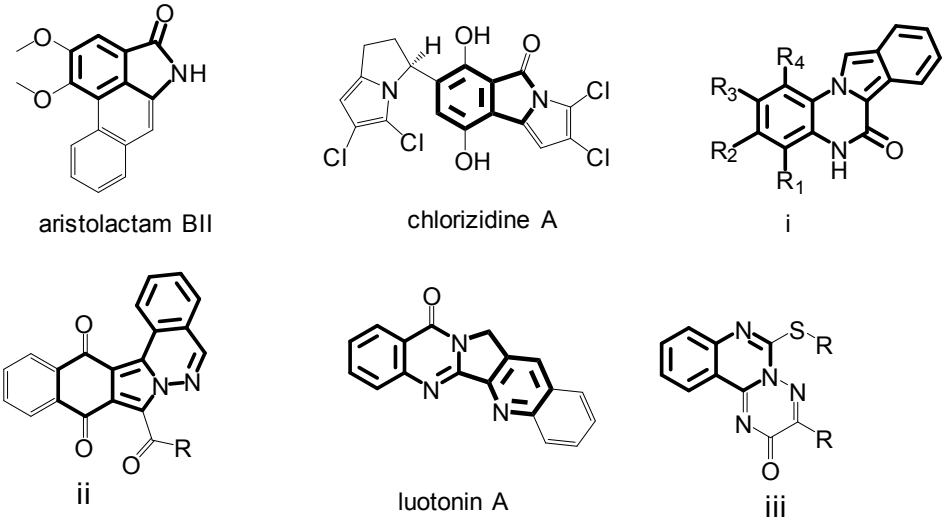


Figure 1. Anticancer agents with isoindole, quinolino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin and [1,2,4]triazino[2,3-*c*]quinazoline fragments as precondition for isoindolo[2,1-a][1,2,4]triazino[2,3-*c*]quinazolines synthesis and evaluation of their biological activity.

(Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography / mass spectrometric system which consists of high performance liquid chromatograph "Agilent 1100 Series" (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL" (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA).

Compounds **1** were obtained according to the described synthetic protocols [9].

General synthetic protocol for 2-R_1-6-R_2-7-R_3-8-R_4-11-R_5-12-R_6-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione (**2.1–2.33**) To suspension of 5 mM of corresponding 3-(2-amino-3-R_2-4-R_3-5-R_4-phenyl)-6-R_1-1,2,4-triazin-5(2*H*)-ones (**1.1–1.23**) in 30 ml of glacial acetic acid 5 mM of 2-formylbenzoic or opianic acid was added. Mixture was refluxed during 4 hours and cooled. Formed solid was filtered off, washed by propanol-2 and dried.

2-methyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14bH)-dione **2.1** Yield 68.35 %; mp 239–240 °C ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.32 (d, J=7.0 Hz, 1H, H-5), 8.17 (d, J=7.2 Hz, 1H, H-11), 8.12 (d, J=7.9 Hz, 1H, H-8), 7.93 (d, J=6.9 Hz, 1H, H-14), 7.87 – 7.62 (m, 3H, H-7, 12, 13), 7.46 (t, J=7.0 Hz, 1H, H-6), 6.94 (s, 1H, H-14b), 2.30 (s, 3H, $\underline{CH_3}$); ^{13}C NMR (126 MHz, DMSO) δ 165.29, 162.49, 154.11, 151.96, 137.43, 135.91, 134.79, 134.76, 133.49, 131.91, 131.39, 129.18, 127.92, 126.21, 124.17, 121.31, 120.22, 72.67, 17.76; EI-MS (m/z(I. rel. %)) 316 (3.3), 276 (9.8), 275 (52.2), 274 (76.9), 247 (19.8), 177 (5), 151 (20.4), 130 (14.7), 129 (14.3), 123 (10.1), 116 (7.6), 105 (32.1), 104 (35.9), 103 (28.8), 102 (100), 96 (7.2), 91 (11.8), 90 (73.8), 89 (31.1), 88 (5.6), 78 (5.3), 77 (67.5), 76 (87.2), 75 (32.9), 69 (6.5), 64 (13.6), 63 (27.7), 62 (9.9), 60 (5.5), 51 (17.8), 50 (10.4), 45 (5.1), 43 (17.9), 42 (14.5); LC-MS m/z=317.0 [M+l]; Anal, calcd. for $C_{18}H_{12}N_4O_2$: C, 68.38; H, 3.84; N, 17.73; O, 10.12; Found: C, 68.39; H, 3.83; N, 17.73; O, 10.14.

2-phenyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.2** Yield 69.98 %; mp 230–232 °C; ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.37 (d, J=7.8 Hz, 1H, H-5), 8.25 (d, J=7.4 Hz, 1H, H-11), 8.22 – 8.09 (m, 3H, H-8, 2-Ph H-2,6), 7.96 (d, J=7.3 Hz, 1H, H-14), 7.87 (t, J=7.4 Hz, 1H, H-7), 7.83 – 7.69 (m, 2H, H-12, 13), 7.58 – 7.38 (m, 4H, H-6, 2-Ph H-3,4,5), 7.06 (s, 1H, H-14b); LC-MS m/z=379.0 [M+l]; Anal, calcd. for $C_{23}H_{14}N_4O_2$: C, 73.01; H, 3.73; N, 14.81; O, 8.46; Found: C, 73.05; H, 3.76; N, 14.85; O, 8.49.

2-(4-ethylphenyl)-3H-isoindolo[2,1-a][1,2,4]triazino[2,3-c]quinazoline-3,10(14bH)-dione **2.3** Yield 85.51 %; mp 253–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.37 (d, J=7.8 Hz, 1H, H-5), 8.25 (d, J=7.4 Hz, 1H, H-11), 8.18 – 8.06 (m, 3H, H-8, 2-Ph H-2,6), 7.97 (d, J=7.4 Hz, 1H, H-14), 7.87 (t, J=7.1 Hz, 1H, H-7), 7.84 – 7.74 (m, 2H, H-12, 13), 7.49 (t, J=7.5 Hz, 1H, H-6), 7.32 (d, J=7.4 Hz, 2H, 2-Ph H-3,5), 7.06 (s, 1H, H-14b), 2.73 (dd, J=14.8, 7.4 Hz, 2H, $\underline{CH_2}CH_3$), 1.30 (t, J=7.5 Hz, 3H, $CH_2\underline{CH_3}$); LC-MS m/z=407.0 [M+l]; Anal, calcd. for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78; O, 7.87; Found: C, 73.91; H, 4.51; N, 13.81; O, 7.92.

2-(4-isopropylphenyl)-3H-isoindolo[2,1-a][1,2,4]triazino[2,3-c]quinazoline-3,10(14bH)-dione **2.4** Yield 85.51 %; mp 253–255 °C; ^{1}H NMR (400 MHz,

dmso-d6+ccl4) δ 8.36 (d, J=7.8 Hz, 1H, H-5), 8.25 (d, J=7.5 Hz, 1H, H-11), 8.17 – 8.06 (m, 3H, H-8), 7.96 (d, J=7.4 Hz, 1H, H-14), 7.87 (t, J=7.4 Hz, 1H, H-7), 7.83 – 7.67 (m, 1H, H-12, 13), 7.49 (t, J=7.6 Hz, 1H, H-6), 7.34 (d, J=8.1 Hz, 1H, 2-Ph H-3,5), 7.05 (s, 1H, H-14b), 2.99 (dt, J=13.6, 6.8 Hz, 1H, $\underline{CH}(CH_3)_2$), 1.31 (d, J=6.8 Hz, 6H, $CH(\underline{CH_3})_2$); LC-MS m/z=421.0 [M+l]; Anal, calcd. for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33; O, 7.61; Found: C, 74.29; H, 4.82; N, 13.37; O, 7.64.

2-(4-(tert-butyl)phenyl)-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.5** Yield 99.69 %; mp 241–243 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.36 (d, J=7.7 Hz, 1H, H-5), 8.26 (d, J=7.4 Hz, 1H, H-11), 8.18 – 8.10 (m, 3H, H-8, 2-Ph H-2, 6), 7.97 (d, J=7.3 Hz, 1H, H-14), 7.88 (t, J=7.3 Hz, 1H, H-7), 7.85 – 7.72 (m, 2H, H-12, 13), 7.50 (d, J=8.2 Hz, 2H, 2-Ph H-3,5), 7.06 (s, 1H, H-14b), 1.38 (s, 9H, $C(CH_3)_3$); LC-MS m/z=435.0 [M+l]; Anal, calcd. for $C_{27}H_{22}N_4O_2$: C, 74.64; H, 5.10; N, 12.89; O, 7.36; Found: C, 74.65; H, 5.12; N, 12.91; O, 7.39.

2-(3,4-dimethylphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.6** Yield 92.00 %; mp 240–242 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.37 (d, J=7.9 Hz, 1H, H-5), 8.25 (d, J=7.5 Hz, 1H, H-11), 8.15 (d, J=8.5 Hz, 1H, H-8), 8.03 – 7.95 (m, J=7.9 Hz, 2H, 2-Ph H-2, 6), 7.92 (d, J=7.4 Hz, 1H, H-14), 7.87 (t, J=7.4 Hz, 1H, H-7), 7.83 – 7.72 (m, 2H, H-12, 13), 7.49 (t, J=7.6 Hz, 1H, H-6), 7.23 (d, J=7.7 Hz, 1H, 2-Ph H-5), 7.05 (s, 1H, H-14b), 2.36 (s, 3H, 4-CH_3), 2.34 (s, 3H, 3-CH_3); LC-MS m/z=407.0 [M+l]; Anal, calcd. for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78; O, 7.87; Found: C, 73.91; H, 4.49; N, 13.82; O, 7.93.

2-(4-methoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.7** Yield 99.25 %; mp 250–252 °C; ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.36 (d, J=7.6 Hz, 1H, H-5), 8.32 – 8.19 (m, 3H, H-11, 2-Ph H-2, 6), 8.15 (d, J=7.8 Hz, 1H, H-8), 7.97 (d, J=7.4 Hz, 1H, H-14), 7.88 (t, J=7.5 Hz, 1H, H-7), 7.85 – 7.72 (m, 2H, H-12, 13), 7.49 (t, J=7.6 Hz, 1H, H-6), 7.12 – 6.89 (m, 3H, H-14b, 2-Ph H-3,5), 3.88 (s, 3H, OCH_3); LC-MS m/z=409.0 [M+l]; Anal, calcd. for $C_{24}H_{16}N_4O_3$: C, 70.58; H, 3.95; N, 13.72; O, 11.75; Found: C, 70.62; H, 3.99; N, 13.76; O, 11.79.

2-(4-ethoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.8** Yield 91.33 %; mp 242–244 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.37 (d, J=7.8 Hz, 1H, H-5), 8.26 (d, J=8.1 Hz, 1H, H-11), 8.22 (d, J=8.4 Hz, 1H, 2-Ph H-2, 6), 8.15 (d, J=8.3 Hz, 1H, H-8), 7.97 (d, J=8.1 Hz, 1H, H-14), 7.88 (t, J=7.8 Hz, 1H, H-7), 7.79 (m, 2H, H-12, 13), 7.49 (t, J=7.4 Hz, 1H, H-6), 7.05 (s, 1H, H-14b), 6.98 (d, J=8.4 Hz, 1H, 2-Ph H-3,5), 4.14 (dd, J=13.4, 6.7 Hz, 2H, $O\underline{CH_2}CH_3$), 1.44 (t, J=6.7 Hz, 1H, $OCH_2\underline{CH_3}$); LC-MS m/z=423.0 [M+l]; Anal, calcd. for $C_{25}H_{18}N_4O_3$: C, 71.08; H, 4.30; N, 13.26; O, 11.36; Found: C, 71.12; H, 4.33; N, 13.31; O, 11.39.

2-(4-fluorophenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione **2.9** Yield 80.66 %; mp 245–247 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.37 (d, J=7.8 Hz, 1H, H-5), 8.31 – 8.26 (m, 2H, 2-Ph H-2, 6), 8.25 (d, J=7.7 Hz, 1H, H-11), 8.15 (d, J=7.8 Hz, 1H, H-8), 7.96 (d, J=7.3 Hz, 1H, H-14), 7.88 (t, J=7.2 Hz, 1H, H-7), 7.85 – 7.72 (m, 2H, H-12, 13), 7.50 (t, J=7.6 Hz, 1H, H-6), 7.25 (t, J=8.7 Hz, 2H, 2-Ph H-3,5), 7.06 (s, 1H, H-14b); LC-MS m/z=397.0 [M+l]; Anal, calcd. for $C_{23}H_{13}FN_4O_2$: C, 69.69; H, 3.31; N, 14.13; O, 8.07; Found: C, 69.72; H, 3.34; N, 14.15; O, 8.11.

8-methyl-2-phenyl-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.10** Yield 81.49 %; mp 292–294 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.29 – 8.12 (m, 3H, H-5, 2-Ph H-2,6), 7.93 (d, J=7.2 Hz, 1H, H-14), 7.85 (t, J=7.2 Hz, 1H, H-7), 7.75 (t, J=7.3 Hz, 1H, H-13), 7.65 (d, J=7.2 Hz, 1H, H-12), 7.54 – 7.40 (m, 4H, H-6, 2-Ph H-3,4,5), 7.02 (s, 1H, H-14b) 2.52 (s, 3H, CH_3); EI-MS (m/z(I. rel. %)) 392 (0.7), 290 (8.1), 289 (53.5), 288 (100), 261 (5.8), 247 (10.1), 219 (10.5), 218 (7.9), 190 (6.2), 116 (5.3), 103 (5.2), 89 (15.7), 63 (7.2); LC-MS m/z=393.0 [M+l]; Anal, calcd. for $C_{24}H_{16}N_4O_2$; C, 73.46; H, 4.11; N, 14.28; O, 8.15; Found: C, 73.46; H, 4.11; N, 14.28; O, 8.15.

7-fluoro-2-phenyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.11** Yield 72.42 %; mp 230–232 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.42 (dd, J=8.6, 6.1 Hz, 1H, H-8), 8.25 (d, J=7.5 Hz, 1H, H-11), 8.17 (d, J=7.7 Hz, 2H, 2-Ph H-2,6), 7.97 (d, J=7.3 Hz, 1H, H-14), 7.93 – 7.83 (m, 2H, H-8, H-13), 7.77 (t, J=7.3 Hz, 1H, H-12), 7.49 (m, 3H, 2-Ph H-3,4, 5), 7.26 (t, J=8.4 Hz, 1H, H-6), 7.07 (s, 1H, H-14b); EI-MS (m/z(I. rel. %)); 396 (0.8), 294 (6.1), 293 (49.0), 292 (100), 265 (12.9); LC-MS m/z=397.0 [M+l]; Anal, calcd. for $C_{23}H_{13}FN_4O_2$; C, 69.69; H, 3.31; F, 4.79; N, 14.13; O, 8.07; Found: C, 69.71; H, 3.33; F, 4.82; N, 14.15; O, 8.09.

7-fluoro-2-(4-methoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.12** Yield 95.36 %; mp 257–259 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.43 (d, 1H, H-5), 8.23 (m, 3H, H-11, 2-Ph H-2, 6), 7.99

(d, 1H, H-14), 7.90 (m, 1H, H-8, 13), 7.79 (t, 2H, H-12), 7.25 (t, 1H, H-6), 7.11 – 6.96 (m, *3*H, H-14b, 2-Ph H-2,6), 3.89 (s, 3H, OCH_3); LC-MS m/z=427.0 [M+l]; Anal, calcd. for $C_{24}H_{15}FN_4O_3$; C, 67.60; H, 3.55; F, 4.46; N, 13.14; O, 11.26; Found: C, 67.63; H, 3.58; F, 4.49; N, 13.17; O, 11.29.

7-fluoro-2-(4-fluorophenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.13** Yield 74.56 %; mp 252–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.53 – 8.34 (m, 1H, H-5), 8.34 – 8.14 (m, 3H, H-11, 2-Ph H-2,6), 7.97 (d, J=7.0 Hz, 1H, H-14), 7.90 (d, J=7.7 Hz, 2H, H-8, 13), 7.78 (t, J=6.9 Hz, 1H, H-12), 7.37 – 7.13 (m, 3H, H-6, 2 Ph H-3,5), 7.07 (s, 1H, H-14b); LC-MS m/z=415.0 [M+l]; Anal, calcd. for $C_{23}H_{12}F_2N_4O_2$; C, 66.67; H, 2.92; F, 9.17; N, 13.52; O, 7.72; Found: C, 66.71; H, 2.98; F, 9.19; N, 13.52; O, 7.75.

6-chloro-2-(4-methoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione **2.14** Yield 83.87 %; mp 260–262 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.49 – 8.08 (m, 5H, H-5,8,11, 2-Ph H-2,6), 8.04 – 7.66 (m, 4H, H-H-7, 12, 13, 14), 7.12 – 6.88 (m, 3H, H-14b, 2-Ph H-3, 5), 3.89 (s, 3H, OCH_3); LC-MS m/z=443.0 [M+l]; Anal, calcd. for $C_{24}H_{15}ClN_4O_3$; C, 65.09; H, 3.41; Cl, 8.00; N, 12.65; O, 10.84; Found: C, 65.11; H, 3.43; Cl, 8.03; N, 12.71; O, 10.87.

6-bromo-2-(4-methoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.15** Yield 91.26 %; mp 263–265 °C; ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.44 (s, 1H, H-5), 8.24 (d, 2H, J=8.0 Hz, 2H, 2-Ph H-2,6), 8.16 – 8.03 (m, 2H, H-8, 11), 8.03 – 7.86 (m, 2H, H-7, 14), 7.86 – 7.66 (m, 2H, H-12, 13), 7.15 – 6.87 (m, 3H, H-14b, 2-Ph H-3,5), 3.88 (s, 3H, OCH_3); LC-MS m/z=488.0 [M+l]; Anal, calcd. for $C_{24}H_{15}BrN_4O_3$: C, 59.15; H, 3.10; Br, 16.40; N, 11.50; O, 9.85; Found: C, 59.19; H, 3.13; Br, 16.43; N, 11.54; O, 9.87.

6-bromo-2-(p-tolyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.16** Yield 82.65 %; mp 271–273 °C; ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.44 (d, J=1.8 Hz (J4), 1H), 8.24 (d, J=7.4 Hz, 1H, H-11), 8.18 – 8.03 (m, 3H, H-8, 2-Ph H-2,6), 8.03 – 7.71 (m, 4H, H-7, 12, 13, 14), 7.29 (d, J=7.4 Hz, 2H, 2-Ph H-3,5), 7.05 (s, 1H, H-14b), 2.45 (s, 3H, CH_3); LC-MS m/z=472.0 [M+l]; Anal, calcd. for $C_{24}H_{15}BrN_4O_2$: C, 61.16; H, 3.21; Br, 16.95; N, 11.89; O, 6.79; Found: C, 61.19; H, 3.23; Br, 16.97; N, 11.91; O, 6.81.

11,12-dimethoxy-2-methyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.17** Yield 67.56 %; mp 274–276 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.31 (d, J=7.2 Hz, 1H, H-5), 8.08 (d, J=7.7 Hz, 1H, H-8), 7.88 – 7.66 (m, 2H, H-7,14), 7.51 – 7.30 (m, 2H, H-6, 13), 6.77 (s, 1H, H-14b), 3.96 (m, 6H, 11-OCH_3, 12-OCH_3, 2.28 (s, 3H, CH_3); EI-MS (m/z(I. rel. %)) 377 (4.7), 376 (21.1), 336 (14.8), 335 (75.5), 334 (100), 320 (39.4), 307 (27.3), 306 (11.5), 305 (20.6), 304 (96.7), 293 (5.4), 290 (12.6), 277 (11.4), 264 (9.8), 263 (12.1), 262 (5), 261 (8.1), 250 (6.2), 249 (13.6), 248 (6), 235 (5.8), 222 (5.7), 221 (15.9), 220 (7.4), 219 (5.1), 193 (9.3), 192 (6.3), 167 (6.5), 165 (6.9), 129 (7.2), 122 (3.3), 107 (7.8), 103 (5.2), 102 (13.4), 77 (7.5), 76 (8.6), 75 (5.6), 63 (5.5); LC-MS m/z=377.0 [M+l]; Anal, calcd. for $C_{20}H_{16}N_4O_4$: C, 63.83; H, 4.29; N, 14.89; O, 17.00; Found: C, 63.85; H, 4.32; N, 14.91; O, 17.03.

11,12-dimethoxy-2-phenyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.18** Yield 93.77 %; mp 253–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.36 (d, J=8.0 Hz, 1H, H-5), 8.19 (d, J=8.4 Hz, 2H, 2-Ph H-2,6), 8.11 (d, J=8.1 Hz, 1H, H-8), 7.88 (d, J=8.4 Hz, 1H, H-14), 7.80 (t, J=7.6 Hz, 1H, H-7), 7.56 – 7.38 (m, 4H, H-6, 2-Ph H-3,4,5), 6.90 (s, 1H, H-14b), 3.97 (m, 6H, 11-OCH_3, 12-OCH_3); EI-MS (m/z(I. rel. %)) 439 (1.7), 438 (4.3), 336 (16.6), 335 (87.5), 334 (100), 320 (6.8), 307 (20.4), 306 (9.3), 305 (18.8), 304 (91.9), 277 (6.2), 264 (5.7), 249 (6.7), 235 (5.3), 89 (5.5), LC-MS m/z=439.0 [M+l]; Anal, calcd. for $C_{25}H_{18}N_4O_4$: C, 68.49; H, 4.14; N, 12.78; O, 14.60; Found: C, 68.52; H, 4.17; N, 12.82; O, 14.61.

11,12-dimethoxy-2-(*p*-tolyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.19** Yield 85.22 %; mp 276–278 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.35 (d, J=7.5 Hz, 1H), 8.10 (d, J=7.6 Hz, 2H, 2-Ph H-2,6), 7.88 (d, J=8.0 Hz, 1H, H-14), 7.79 (t, J=7.0 Hz, 1H, H-7), 7.50 – 7.45 (m, 2H, H-6, H-13), 7.29 (d, J=7.5 Hz, 2H, 2-Ph H-3, 5), 6.88 (s, 1H, H-14b), 3.97 (m, 6H, 11-OCH_3, 12-OCH_3), 2.44 (s, 3H, CH_3); LC-MS m/z=453.0 [M+l]; Anal, calcd. for $C_{26}H_{20}N_4O_4$: C, 69.02; H, 4.46; N, 12.38; O, 14.14; Found: C, 69.04; H, 4.49; N, 12.41; O, 14.15.

2-(4-ethylphenyl)-11,12-dimethoxy-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.20** Yield 92.43 %; mp 276–278 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.35 (d, J=7.7 Hz, 1H, H-5), 8.12 (m, 3H, H-8, 2-Ph H-2,6), 7.88 (d, J=8.3 Hz, 1H, H-14), 7.79 (t, J=7.7 Hz,1H, H-7), 7.60 – 7.40 (m, 2H, H-6, H-13), 7.32 (d, J=7.4 Hz, 2H, 2-Ph H-3,5), 6.89 (s, 1H, H-14b), 3.97 (m, 6H, 11-OCH_3, 12-OCH_3), 2.73 (dd, J=15.9, 7.3 Hz, 2H, $\underline{CH_2}CH_3$), 1.30 (m, J=7.3 Hz, 3H,

$\underline{CH_2}CH_3$); LC-MS m/z=467.0 [M+l]; Anal, calcd. for $C_{27}H_{22}N_4O_4$: C, 69.52; H, 4.75; N, 12.01; O, 13.72; Found: C, 69.54; H, 4.79; N, 12.04; O, 13.75.

2-(4-isopropylphenyl)-11,12-dimethoxy-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.21** Yield 80.78 %; mp 266–268 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.35 (d, J=7.8 Hz, 1H, H-5), 8.18 – 8.04 (m, 3H, 2-Ph H-2, 6, H-8), 7.88 (d, J=8.3 Hz, 1H, H-14), 7.79 (t, J=7.6 Hz, 1H, H-7), 7.48 (m, 2H, H-6, 13), 7.34 (d, J=7.9 Hz, 2H, 2-Ph H-3,5), 6.89 (s, 1H, H-14b), 3.98 (m, 6H, 11-OCH_3, 12-OCH_3), 3.02 – 2.91 (m, 1H, $\underline{CH}(CH_3)_2$), 1.29 (d, J=6.8 Hz, 6H, $CH(\underline{CH_3})_2$); LC-MS m/z=481.0 [M+l]; Anal, calcd. for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66; O, 13.32; Found: C, 70.02; H, 5.053; N, 11.69; O, 13.35.

2-(4-(tert-butyl)phenyl)-11,12-dimethoxy-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.22** Yield 94.17 %; mp 268–270 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.33 (d, J=7.5 Hz, 1H, H-5), 8.21 – 8.01 (m, 3H, 2-Ph H-8, H-2,6), 7.89 (d, J=8.2 Hz, 1H, H-14), 7.78 (t, J=7.2 Hz, 1H, H-7), 7.60 – 7.33 (m, 3H, H-H-6, 2-Ph H-3,5), 6.88 (s, 1H, H-14b), 3.97 (m, 6H, 11-OCH_3, 12-OCH_3), 1.38 (s, 3H, $C(CH_3)_3$); LC-MS m/z=495.0 [M+l]; Anal, calcd. for $C_{29}H_{26}N_4O_4$: C, 70.43; H, 5.30; N, 11.33; O, 12.94; Found: C, 70.45; H, 5.33; N, 11.37; O, 12.98.

2-(3,4-dimethylphenyl)-11,12-dimethoxy-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.23** Yield 93.48 %; mp 253–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.34 (d, J=7.3 Hz, 1H, H-5), 8.10 (d, J=7.0 Hz, 1H, H-8), 7.99 (s, 1H, 2-Ph H-2), 7.96 – 7.82 (m, 2H, H-14, 2-Ph H-6), 7.79 (t, J=7.3 Hz, 1H, H-7), 7.63 – 7.35 (m, 2H, H-6, 2-Ph H-5), 7.23 (d, J=7.7 Hz, 1H, H-13), 6.88 (s, 1H, H-14b), 3.97 (m, 6H, 11-OCH_3 12-OCH_3), 2.37 (s, 3H, 2-Ph 4-CH_3), 2.34 (s, 3H, 2-Ph 3-CH_3); LC-MS m/z=467.0 [M+l]; Anal, calcd. for $C_{27}H_{22}N_4O_4$: C, 69.52; H, 4.75; N, 12.01; O, 13.72; Found: C, 69.53; H, 4.75; N, 12.06; O, 13.78.

2-(4-ethoxyphenyl)-11,12-dimethoxy-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.24** Yield 90.76 %; mp 269–271 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.35 (d, J=7.8 Hz, 1H, H-5), 8.22 (d, J=8.0 Hz, 2H, 2-Ph H-2,6), 8.10 (d, J=7.8 Hz, 1H, H-8), 7.89 (d, J=7.9 Hz, 1H, H-14), 7.79 (t, 1H, H-7), 7.47 (d, 1H, H-13), 6.98 (d, J=8.1 Hz, 2H, 2-Ph H-3,5), 6.88 (s, 1H, H-14b), 4.14 (m, 2H, $O\underline{CH_2}CH_3$), 3.98 (s, 6H, 11-OCH_3, 12-OCH_3), 1.45 (s, 3H, $OCH_2\underline{CH_3}$); LC-MS m/z=483.00 [M+l]; Anal, calcd. for $C_{27}H_{22}N_4O_5$: C, 67.21; H, 4.60; N, 11.61; O, 16.58; Found: C, 67.25; H, 4.64; N, 11.65; O, 16.61.

2-(4-fluorophenyl)-11,12-dimethoxy-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione **2.25** Yield 92.59 %; mp 253–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.35 (d, J=7.4 Hz, 1H, H-5), 8.30 (t, J=6.5 Hz, 2H, 2-Ph H-2,6), 8.10 (d, J=7.3 Hz, 1H, H-8), 7.88 (d, J=7.8 Hz, 1H, H-14), 7.840 (t, 1H, J=7.3 Hz, H-7), 7.59 – 7.39 (m, 2H, H-6, 13), 7.24 (t, J=7.9 Hz, 2H, 2-Ph 3, 5), 6.89 (s, 1H, H-14b, 3.97 (m, 6H, 11-OCH_3, 12-OCH_3); LC-MS m/z=457.0 [M+l]; Anal, calcd. for $C_{25}H_{17}FN_4O_4$: C, 65.79; H, 3.75; F, 4.16; N, 12.28; O, 14.02; Found: C, 65.82; H, 3.77; F, 4.19; N, 12.31; O, 14.05.

11,12-dimethoxy-8-methyl-2-phenyl-*3H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.26** Yield 86.04 %; mp 276–279 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.24 – 8.11 (m, *3*H, H-5, 2-Ph H-2,6), 7.86 (d, J=8.2 Hz, 1H, H-14), 7.64 (d, J=7.3 Hz, 1H, H-7), 7.55 – 7.40 (m, 5H, H-6, 13, 2-Ph H-3, 4, 5), 6.86 (s, 1H, H-14b), 3.96 (s, 3H, 4-OCH_3), 2.48 (s, 3H, 3-OCH_3); EI-MS (m/z(I. rel. %)) 454 (7.6), 453 (26), 350 (54.3), 349 (94.2), 348 (100), 335 (20.7), 334 (75.9), 332 (11.2), 331 (18.8), 330 (8.4), 321 (34.6), 320 (15.8), 318 (56.6), 316 (6), 307 (27.5 %), 306 (12.4), 304 (12.5), 300 (5.5), 290 (8.2), 277 (27.2), 263 (20.9), 249 (12), 247 (7.8), 236 (6.8), 235 (16.7), 233 (5.8), 221 (7.1), 219 (6.6), 218 (5.6), 207 (7.4), 206 (10.5), 193 (5.4), 192 (7.9), 190 (6.1), 174 (9.2), 167 (8.6), 166 (11.7), 165 (25.4), 153 (9.4), 152 (8), 146 (5.3), 145 (9.3), 140 (5.7), 139 (7.5), 131 (7.2), 130 (6.9), 122 (9.9), 121 (7.7), 119 (5.1), 118 (7), 117 (12.9), 116 (29), 115 (8.7), 114 (5.2), 107 (22.5), 106 (10), 105 (8.8), 104 (26.8), 103 (39.9), 102 (11.9), 92 (6.2), 91 (10.3), 90 (17.1), 89 (69.9), 79 (13.8), 78 (19.7), 77 (35.2), 76 (35.9), 75 (9.6), 65 (14.9), 63 (37.9), 52 (6.3), 51 (17.3 %) LC-MS m/z=453.0 [M+l]; Anal, calcd. for $C_{26}H_{20}N_4O_4$; C, 69.02; H, 4.46; N, 12.38; O, 14.14 Found: C, 69.05; H, 4.47; N, 12.39; O, 14.19.

6-chloro-11,12-dimethoxy-2-methyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14bH)-dione **2.27** Yield 80.66 %; mp 268–271 °C; ^{1}H NMR (400 MHz, dmso-d6) δ 8.25 (s, 1H, H-5), 8.09 (d, J=8.6 Hz, 1H, H-8), 7.90 – 7.67 (m, 2H, H-7, 14), 7.42 (d, J=8.3 Hz, 1H, H-13), 6.77 (s, 1H, H-14b), 3.96 (m, 6H, 4-OCH_3, 3-OCH_3), 2.29 (s, 3H, 2-CH_3); LC-MS m/z=411.0 [M+l]; Anal, calcd. for $C_{20}H_{15}ClN_4O_4$; C, 58.47; H, 3.68; Cl, 8.63; N, 13.64; O, 15.58; Found: C, 58.49; H, 3.69; Cl, 8.67; N, 13.69; O, 15.61.

6-chloro-11,12-dimethoxy-2-phenyl-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.28** Yield 85.52 %; mp 281–283 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.29 (d, J=2.3 Hz, 1H, H-5), 8.19 (d, J=7.7 Hz, 1H, 2-Ph H-2,6), 8.12 (d, J=8.6 Hz, 1H, H-8), 7.88 (d, J=8.2 Hz, 1H, H-14), 7.79 (dd, J=8.7, 2.3 Hz, 1H, H-7), 7.58 – 7.42 (m, 4H, H-13, 2-Ph H-3, 4, 5), 6.90 (s, 1H, 14b), 3.97 (s, 6H, 3-OCH_3, 4-OCH_3); LC-MS m/z=472.0 [M+l]; Anal, calcd. for $C_{25}H_{17}ClN_4O_4$; C, 63.50; H, 3.62; Cl, 7.50; N, 11.85; O, 13.53; Found: C, 63.50; H, 3.62; Cl, 7.50; N, 11.85; O, 13.53.

6-chloro-2-(4-fluorophenyl)-11,12-dimethoxy-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.29** Yield 64.58 %; mp 277–279 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.36 – 8.18 (m, *3*H, H-5, 2-Ph H-2,6), 8.11 (d, J=8.7 Hz, 1H, H-8), 7.87 (d, J=8.3 Hz, 1H, H-14), 7.79 (dd, J=8.7, 2.3 Hz, 1H, H-7), 7.47 (d, J=8.3 Hz, 1H, H-13), 7.24 (t, J=8.7 Hz, 2H, 2-Ph H-3,5), 6.89 (s, 1H, H-14b), 3.97 (s, 6H, 4-OCH_3, 3-OCH_3); LC-MS m/z=491.0 [M+l]; Anal, calcd. for $C_{25}H_{16}ClFN_4O_4$; C, 61.17; H, 3.29; Cl, 7.22; F, 3.87; N, 11.41; O, 13.04; Found: C, 61.19; H, 3.31; Cl, 7.25; F, 3.91; N, 11.44; O, 13.07.

6-bromo-11,12-dimethoxy-2-methyl-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.30** Yield 66.98 %; mp 261–263 °C; ^{1}H NMR (400 MHz, dmso-d6) δ 8.39 (s, 1H, H-5), 8.03 (d, J=8.5 Hz, 1H, H-8), 7.89 (d, J=8.2 Hz, 1H, H-7), 7.78 (d, J=8.0 Hz, 1H, H-14), 7.42 (d, J=8.4 Hz, 1H, H-12), 6.76 (s, 1H, H-14b), 3.96 (m, 6H, 3-OCH_3, 4-OCH_3), 2.29 (s, 3H, 2-CH_3); LC-MS m/z=456.0 [M+l]; Anal, calcd. for $C_{20}H_{15}BrN_4O_4$; C, 52.76; H, 3.32; Br, 17.55; N, 12.31; O, 14.06; Found: C, 52.76; H, 3.32; Br, 17.55; N, 12.31; O, 14.06.

6-bromo-11,12-dimethoxy-2-phenyl-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14*bH*)-dione **2.31** Yield 83.09 %; mp 280–283 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.43 (s, 1H, H-5), 8.25 – 8.10 (m, 2H, 2-Ph H-2, 6), 8.06 (d, J=8.6 Hz, 1H, H-8), 7.92 (d, J=6.9 Hz, 1H, H-7), 7.88 (d, J=8.7 Hz, 1H, H-14), 7.59 – 7.40 (m, 3H, H-13, 2-Ph H-3, 4, 5), 6.90 (s, 1H, H-14b), 3.97 (m, 6H, 4-OCH_3 3-OCH_3); 517 (1.9), 516 (2.4), 415 (8.9), 414 (15.7), 413 (15.6 %), 412 (15.3), 400 (6.4), 398 (8.8), 387 (8.4), 385 (100.0), 384 (30.5), 383 (6.1), 382 (28.6), 300 (6.2), 299 (10.5), 170 (7.9), 168 (7.1), 166 (5.1), 165 (14.6), 164 (10.2), 151 (7.3), 150 (4.3); LC-MS m/z=518.0 [M+l]; Anal, calcd. for $C_{26}H_{20}N_4O_4$; C, 58.04; H, 3.31; Br, 15.45; N, 10.83; O, 12.37; Found: C, 58.07; H, 3.33; Br, 15.47; N, 10.87; O, 12.39.

6-bromo-11,12-dimethoxy-2-(4-methoxyphenyl)-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione **2.32** Yield 98.85 %; mp 278–280 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.42 (s, 1H, H-5), 8.24 (d, J=8.5 Hz, 2H, 2-Ph H-2,6), 8.05 (d, J=8.7 Hz, 1H, H-8), 7.95 – 7.83 (m, 2H, H-7, 14), 7.49 (d, J=8.9 Hz, 1H, H-13), 7.01 (d, J=8.6 Hz, 2H, 2-Ph H-3,5), 6.88 (s, 1H, H-14b), 3.97 (s, 3H, 4-OCH_3), 3.89 (s, 3H, -OCH_3); LC-MS m/z=548.0 [M+l]; Anal, calcd. for $C_{26}H_{19}BrN_4O_5$; C, 57.05; H, 3.50; Br, 14.60; N, 10.24; O, 14.61; Found: C, 57.07; H, 3.53; Br, 14.63; N, 10.27; O, 14.65.

6-bromo-2-(4-fluorophenyl)-11,12-dimethoxy-3H-isoindolo[2,1-*a*][1,2,4]triazino[2,3-c]quinazoline-3,10(14*bH*)-dione **2.33** Yield 99.3 %; mp 253–255 °C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.42 (d, J=1.7 Hz, 1H, H-5), 8.35 – 8.21 (m, 2H, 2-Ph H-2,6), 8.06 (d, J=8.8 Hz, 1H, H-8), 7.92 (dd, J=9.1, 1.7 Hz, 1H, H-7), 7.87 (d, J=8.8 Hz, 1H, H-14), 7.47 (d, J=8.3 Hz, 1H, H-13), 7.25 (t, J=8.7 Hz, 2H, 2-Ph H-3,5), 6.89 (s, 1H, H-14b), 3.97 (s, 6H, 4-OCH_3, 3-OCH_3); LC-MS m/z=534.0 [M+l]; Anal, calcd. for $C_{25}H_{16}BrFN_4O_4$; C, 56.09; H, 3.01; Br, 14.93; F, 3.55; N, 10.47; O, 11.95; Found: C, 56.09; H, 3.01; Br, 14.93; F, 3.55; N, 10.47; O, 11.95.

2-(2-oxo-3-phenyl-6,7-dihydro-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)benzoic acid (**3.1**) was added to suspension of 5 mM of 3-(2-aminophenyl)-6-penyl-1,2,4-triazin-5(2H)-one (**1.2**) in 30 ml of dioxane 5 mM of 2-formylbenzoic. Mixture was refluxed during 3 hours and cooled. Formed solid was filtered off, washed by propanol-2 and dried.

2-(2-oxo-3-phenyl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)benzoic acid **3.1** Yield 37.9 %; mp 281–283 °C, ^{1}H NMR (400 MHz, dmso-d6) δ 13.50 (s, 1H, COOH), 8.05 – 7.99 (m, 2H, H-11, 6-Ph H-3), 7.97 (d, J=7.3 Hz, 2H, 3-Ph H-2,6), 7.73 (s, 1H, NH), 7.65 – 7.28 (m, 7H, H-9, 3-Ph H-3,4,5, 6-Ph H-4,5,6), 6.96 (d, J=7.7 Hz, 1H, H-8), 6.89 (t, J=7.7 Hz, 1H, H-10); LC-MS m/z=397.0 [M+l]; Anal, calcd. for $C_{23}H_{16}N_4O_3$; C, 69.69; H, 4.07; N, 14.13; O, 12.11; Found: C, 69.72; H, 4.09; N, 14.16; O, 12.16.

X-ray experimental part

The colourless crystals of **2.1** ($C_{18}H_{12}N_4O_2 \cdot C_2H_4O_2$) are monoclinic. At 293 K a=14.773(2), b=16.527(3), c=7.270(2) Å, β=94.81(2)°, V=1768.7(6) Å^3, M_r=376.37, Z=4, space group $P2_1/c$, d_{calc}= 1.413 g/cm^3, μ(MoK_α)=0.101 mm^{-1}, F(000)=784. Intensities of 13668 reflections (3117 independent, R_{int}=0.095) were measured on the "Xcalibur-3" diffractometer (graphite monochromated MoK_α

radiation, CCD detector, ω-scaning, $2\Theta_{max}$=50°). The structure was solved by direct method using SHELXTL package [18]. Positions of the hydrogen atoms were located from electron density difference maps and refined by "riding" model with U_{iso}=nU_{eq} (n=1.5 for methyl group and n=1.2 for other hydrogen atoms) of the carrier atom. Hydrogen atom taking part in the formation of the hydrogen bond is refined using isotropic approximation. Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms using 3072 reflections was converged to wR_2=0.137 (R_1=0.054 for 1572 reflections with F>4σ(F), S=0.897). The final atomic coordinates, and crystallographic data for molecule **2.1** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 1062193).

Molecular modeling

Receptor and ligand preparation for flexible docking. The crystal structure of human protein kinase $FGFR_1$ was obtained from the Brookhaven Protein Data Bank (PDB ID: 3GQI) [19]. The catalytic subunit has been extracted from the PDB file, and the ligand has been removed from the $FGFR_1$-phosphomethylphosphonic acid adenylate ester complex. The PDB file of $FGFR_1$ catalytic subunit was converted to PDBQT format via MGLTools 1.5.6 [16]. Receptor grid maps were prepared using MGLTools 1.5.6 and AutoGrid 4.2.6 [16].

Ligand files in PDBQT format were generated via Vega ZZ 3.0.5.12 (command line) [20] using force field AUTODOCK with further removing of nonpolar hydrogens.

Flexible docking

Autodock 4.2.6 program (La Jolla, CA) has been used for receptor-ligand flexible docking [16]. Autodock input parameters have been set as the following: maximum translation jump per step – 2 Å, maximum orientation step size for the angular component w of quaternion – 50°, maximum dihedral step size – 50°, number of the torsional degrees of freedom – 2, rms deviation tolerance for cluster analysis – 2 Å, optional external grid energy – 1000, number of individuals in the population – 300, maximum number of energy evaluations – 1 000 000, maximum number of generations – 27 000, number of top individuals that are guaranteed to survive into the next generation – 1, the probability that a particular gene is mutated – 0.02, crossover rate – 0.8, number of preceding generations to take into consideration when deciding the threshold for the worst individual in the current population – 10, alpha parameter in Cauchy distribution – 0, beta parameter in Cauchy distribution – 1.

Visual analysis

Visual analysis of the complexes of the ligands with amino acid residues of ATP-binding site of protein kinase FGFR1 was performed using the program Discovery Studio Visualizer 4.0 [21].

Biochemical testing

Compounds were tested using in vitro kinase assay [22]. Each test was done in a total reaction volume of 30 µl, containing 6 µl 5^x buffer solution (10 mM MOPS; pH 7.2; 0.1 mM $NaVO_4$, 0.2 mg/ml BSA; 0.2 mM EDTA; 0.002 % Brij 35 and 0.02 % β-mercaptoethanol), 2.5 µl of IGF-IRtide substrate solution (4.0 µg/reaction), 10.5 µl of H_2O and 0.075 µl of recombinant $FGFR_1$ catalytic subunit expressed in insect cells Sf21 (Upstate Millipore, cat. 14582) (10.5 mU, ~7.35 ng/reaction), 1 µl of inhibitor DMSO solution in varying concentrations, and 10 µl of ATP solution 3^x (150 µM ATP; 30 mM $Mg(CH_3COO)_2$; 1.5 mM HEPES) with γ-labeled ^{32}P ATP added, diluted to specific activity 100 µCi/µM. The final contration of ATP in reaction volume was 50 µM. Incubation time was 20 min at 30 °C. The reaction was stopped by adding 8 µl of 0.5 M orthophosphoric acid and the total reaction mixture was loaded on the 18x18 mm filter squares of the cellulose phosphate paper p31 or p81 (Whatman). Filters were washed three times for 5 minutes with 0.075 M orthophosphoric acid on the shaker platform, air-dried at room temperature, placed in scintillate vials and counted by PerkinElmer Tri-Carb 2800-TR Liquid Scintillation Analyzer. Percent inhibition was calculated as ratio of substrate-incorporated radioactivity in the presence of inhibitor to the radioactivity incorporated in control reactions, i.e. in the absence of any inhibitor but with DMSO as background. Serial dilutions of inhibitor stock solution were used to determine its IC_{50} concentration.

IC_{50} calculation. All compounds were preliminary "screened" for activity with $FGFR_1$ at 33 µM concentration. For those inhibitors decreasing activity of protein kinase $FGFR_1$ for more than 75 % in comparison with the background DMSO probes, the residual activity of enzyme was determined at 10 µM inhibitor concentration. For compounds decreasing activity of protein kinase $FGFR_1$ for more than 55 % under this inhibitor concentration the IC_{50} values were obtained.

The titration curves were built in coordinates of lg[C]µM and CPM. Lg[C] for mean value of CPM in the point half between upper and lower asymptotes was determined. The inverse logarithm of inhibitor concentration in this point was taken as IC_{50} value [µM].

RESULTS AND DISCUSSION.

Chemistry

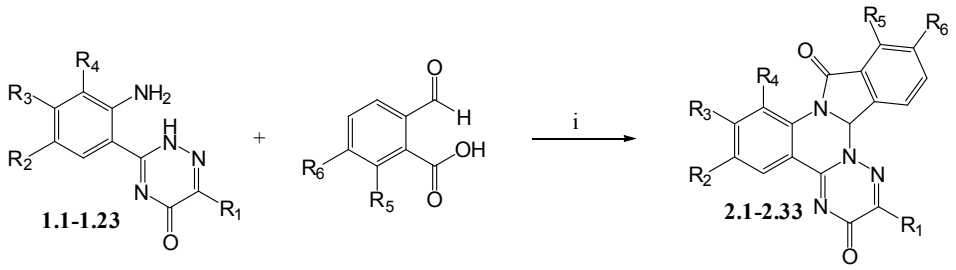
As initial compounds we used anilines **1.1–1.23** [9] which were previously described as effective 1,5-binuclephilic "scaffolds" useful for formation of various substituted [1,2,4]triazino[2,3-*c*]quinazolines. For modification of the mentioned precursors aimed at the synthesis of target compounds we used as the reagents 2-formylbenzoic and 6-formyl-2,3-dimethoxybenzoic (opianic) acids. Experimental data showed that refluxing of starting substances in glacial acetic during 4 h. resulted in corresponding substituted isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*] quinazoline-3,10(14b*H*)-diones with good yields (Scheme 1).

Considering the multistep nature of presented reaction we were interested in structure of its intermediates. Thus, we decided to conduct interaction between 1.2 and 2-formylbenzoic acid in more mild conditions. According to LC-MS and ^{1}H NMR data as a result of above mentioned interaction was yielded **3.1** (Scheme 2). Further refluxing of this compound in acetic acid during 4 hour produced described above condensed derivative **2.2**, what as we supposed, proved the fact that 2-R_5-3-R_6-6-(3-R_1-8-R_4-9-R_3-10-R_2-2-oxo-6,7-dihydro-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)benzoic acid was the intermediate of the reaction presented at Scheme 1.

Purity of synthesized compounds has been proven by LC-MS (APCI) method, the structure has been established by combination of several physicochemical methods including ^{1}H and ^{13}C NMR, IR-, mass-(EI) – spectrometry.

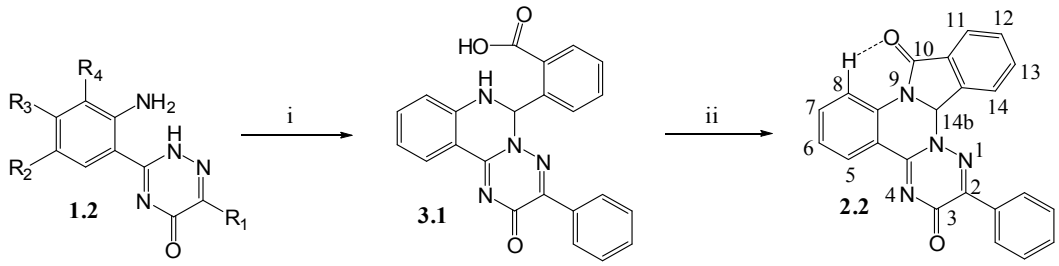
^{1}H NMR spectra of compounds **2.1–2.33** were characterized by signal of abnormally deshielded proton at 14b sp^3 – hybridized carbon atom which were observed at 6.76–7.07 ppm. Signals of protons at position 8 were also characteristic considering their location at lower field (8.15–7.90 ppm) comparing to previously described triazinoquinazoline systems [10]. Mentioned above specific chemical shifts may be explained by hydrogen bond formation between hydrogen at position 8 and oxygen at position 10, which additionally proves formation of isoindolotriazinoquinazoline system. All other signals of mentioned above heterocyclic system and substituents at position 2 were also present.

^{13}C NMR spectra also corresponded with proposed for compounds **2.1–2.33** structures. Thus, in ^{13}C NMR spectra of compound **2.1** was characteristic the signal of 14b carbon atom which was observed at 72.67 ppm. The studying of mass-spectra (EI) of compounds **2.1**, **2.10**, **2.11**, **2.17**, **2.18**, **2.26**, **2.31** allowed us to estimate, that the main directions of synthesized compounds fragmentation were caused by degradation of triazine cycle. Thus, signals of fragmental ions which were formed as result of N1-N15 and C2-C3, N1-N15 and C3-N4, N1-N15 and N4-N5 bonds cleavage were observed.



i - acetic acid, 4 h., refluxing; R_1 = Me, Ph, 4-MePh, 4-EtPh, 4-i-PrP, 4-t-Bu-Ph, 3,4-Me_2Ph, 4-MeOPh, 4-EtOPh, 4-FPh; R_2 = H, Cl, Br; R_3 = H, F, R_4 = H, Me; R_5 = H, OMe; R_6 = H, OMe

Scheme 1. Synthesis of substituted isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14b*H*)-diones.



i - 2-formylbenzoic acid, dioxane, 3 h., refluxing; ii - acetic acid, 4 h., refluxing;

Scheme 2. Synthesis of intermediate 3.1 and its cyclization.

In ^{1}H NMR spectra of compound 3.1 were characteristic the signals of carboxyl group proton (13.50 ppm) and N(7)H fragment (7.73 ppm).

In spite of full accordance of spectral data with proposed structure of compounds **2.1–2.31** we decided to carry out X-ray verification of the structure for compound **2.1** (Figure 2). The compound **2.1** exists as monosolvate with acetic acid in the crystal phase. The partial saturated heterocycle adopts an intermediate between twist-boat and sofa conformation with following puckering parameters [11]: S=0.58, Θ=47.7°, Ψ=23.6°. Deviations of the N3 and C3 atoms from the mean plane of the remaining atoms of the ring are 0.23 Å and 0.65 Å, respectively. In the crystal the molecules **2.1** and solvate acid molecules are bonded by the O(2S)-H…O(1)' (1-x, 1-y, 1-z) (H…O 1.70 Å O-H…O 175°) intermolecular hydrogen bond. This results in the shortening of the C1-O1 bond up to 1.239(3) Å as compared with its mean value [12] 1.210 Å. Molecules **2.1** also form the columns along the [0 0 1] crystallographic direction due to stacking interactions between C11…C16 aromatic rings with the distance between π-systems about 3.4 Å.

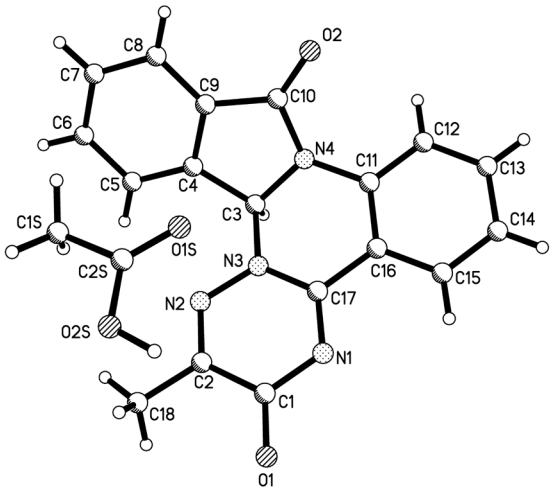


Figure 2. Molecular structure of compound **2.1** according to X-ray diffraction study.

Biology

Anticancer activity

Compounds **2.1**, **2.2**, **2.8**, **2.10**, **2.12**, **2.13**, **2.14**, **2.26**, **2.27**, **2.28**, **2.29**, **2.31**, **2.32** and **2.33** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol, which was described elsewhere [13–15]. The compounds were evaluated at one dose primary anticancer assay relative to approximately 60 cell lines (concentration 10 μM). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Test agents were then added at a single concentration and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested agent were reported as the percent growth of the treated cells comparing to the untreated control cells. The screening results are shown in Table 1.

The anticancer assay data showed that some of studied compounds revealed significant anticancer activity against certain cell lines. The most sensitive were cell lines of renal cancer (ACHN/RC, CAKI-1/RC, UO-31/RC, TK-10/RC, RXF 393/RC).

The most active were compounds **2.12** (mean growth 71.14 %, range of growth 1.97–105.02 %), **2.14** (mean growth 71.24 %, range of growth 24.24–110.60 %). The above mentioned substances were characterized by the broadest spectrum of activity and most significant level of growth inhibition. The most sensitive towards growth inhibitory activity of compound **2.12** were cells of non-small cell lung cancer HOP-62, however, we noted that this compound demonstrated significant activity against almost all renal cancer cell lines. Compound **2.14** was also active against renal cancer cell lines CAKI-1/RC (36.77 %) and TK-10/RC (35.85 %), but the most sensitive to this compound was leukemia SR cell line (24.24 %). Moreover we noted expressed growth inhibitory activity of compound **2.26** against CAKI-1 renal cancer cell line (13.94 %). Another sensitive to the synthesized compounds was SF-539/CNSC cell line.

Therefore, compounds **2.12**, **2.14**, **2.29** and **2.32** significantly inhibited growth of several cancer cell lines.

$FGFR_1$ kinase inhibitory activity

To identify inhibitors of protein kinase $FGFR_1$ among synthesized compounds, we have performed receptor-oriented virtual screening of the library containing 1520 compounds. Autodock software was used to conduct receptor-ligand flexible docking [16]. After the docking followed by visual inspection of the best-scored complexes, six compounds were taken for in vitro kinase assay.

Selected compounds were tested for activity toward $FGFR_1$ at 33 μM concentration. Five compounds which decreased activity of $FGFR_1$ for more than 75 % were tested against protein kinase at 10 μM concentration. For three compounds decreasing activity of $FGFR_1$ for more than 55 % at 10 μM concentration, the IC_{50} values were calculated (Table 2).

Table 1 – **Percentage of *in vitro* tumor cell lines growth at 10 μM of synthesized compounds**

Test compounds	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %*
2.1	105.59	62.63 - 130.00	62.63 (A498/RC)
2.2	97.01	53.53 - 126.73	76.15 (NCI-H522/nscLC), 75.11 (HCT-116/ColC), 63.51 (HT29/ColC), 77.85 (IGROV1/OV), *53.53 (A498/RC)*, 73.77 (UO-31/RC)
2.8	105.40	85.75 - 133.04	87.88 (NCI-H522/nscLC), 86.01 (SF-539/CNSC), 85.75 (UO-31/RC)
2.10	87.20	46.59 - 111.55	*66.59 (CCRF-CEM/L, 64.78 (MOLT-4/L)*, 76.62 (RPMI-8226/L), 58.27 (SR/L), 79.93 (NCI-H226/nscLC), 79.69 (HCT-15/ColC), 71.11 (HT29/ColC), 77.63 (SF-295/CNSC), 79.11 (UACC-62/M), 65.36 (A498/RC), 79.08 (ACHN/RC), *46.59 (CAKI-1/RC)*, 78.74 (TK-10/RC), 70.56 (UO-31/RC), *53.26 (MCF7/BC)*, 67.81 (T-47D/BC), 70.43 (MDA-MB-468/BC)
2.12	71.14	-1.97 - 105.02	64.24 (SR/L), 71.64 (A549/ATCC/nscLC), *-1.97 (HOP-62/nscLC)*, *35.84 (NCI-H226/nscLC)*, 69.15 (NCI-H460/nscLC), 69.72 (HCT-116/ColC), 75.25 (HCT-15/ColC), 69.65 (HT29/ColC), *46.68 (SF-268/CNSC)*, 58.30 (SF-295/CNSC), 61.55 (SF-539/CNSC), 64.60 (SNB-19/CNSC), *41.48 (SNB-75/CNSC)*, 72.32 (U251/CNSC), *56.92 (LOX IMVI/M)*, 61.20 (MALME-3M/M), 77.65 (SK-MEL-28/M), 76.01 (UACC-257/M), 69.25 (IGROV1/OV), 63.25 (OVCAR-3/OV), *56.14 (OVCAR-4/OV)*, *47.99 (OVCAR-8/OV)*, 78.41 (NCI/ADR-RES/OV), 37.30 (SK-OV-3/OV), *51.60 (786-0/RC)*, *58.32 (ACHN/RC)*, *60.44 (CAKI-1/RC)*, *52.85 (RXF 393/RC)*, 79.03 (SN12C/RC), 61.51 (TK-10/RC), 67.62 (PC-3/PC), 72.94 (DU-145/PC), 58.37 (MCF7/BC), 73.83 (MDA-MB-231/ATCC/BC), 60.60 (T-47D/BC), 73.47 (MDA-MB-468/BC)
2.13	88.68	52.19 - 118.88	70.59 (CCRF-CEM/L), 78.49 (MOLT-4/L), 70.56 (RPMI-8226/L), 74.03 (SR/L), 76.51 (HCT-116/ColC), *61.10 (HCT-15/ColC)*, 60.97 (HT29/ColC), 81.21 (SF-295/CNSC), 78.87 (SNB-19/CNSC), 77.57 (UACC-62-/M), 73.54 (IGROV1/OV), *52.19 (UO-31/RC)*, 75.66 (PC-3/PC), 75.03 (MCF7/BC), 73.70 (T-47D/BC)
2.14	71.24	24.24 - 110.60	*57.50 (CCRF-CEM/L)*, 62.95 (K-562/L), 77.16 (MOLT-4/L), *24.24 (SR/L)*, 70.23 (A549/ATCC/nscLC), 60.99 (HOP-62/nscLC), 77.60 (NCI-H226/nscLC), 78.67 (NCI-H23/nscLC), 79.09 (NCI-H322M/nscLC), *52.61 (HCT-116/ColC)*, 77.14 (HCT-15/ColC), 62.69 (HT29/ColC), 76.14 (KM12/ColC), 64.34 (SF-268/CNSC), *35.26 (SF-539/CNSC)*, 73.45 (SNB-75/CNSC), *44.14 (LOX IMVI/M)*, *53.26 (MALME-3M/M)*, 73.51 (M14/M), 64.90 (MDA-MB-435/M), 79.28 (SK-MEL-2/M), 71.07 (SK-MEL-28/M), 74.13 (SK-MEL-5/M), 75.76 (UACC-257/M), 79.52 (UACC-62/M), 74.09 (OVCAR-3/OV), 67.53 (OVCAR-4/OV), 64.95 (OVCAR-8/OV), 75.23 (NCI/ADR-RES/OV), 77.35 (SK-OV-3/OV), 73.14 (ACHN/RC), *36.77 (CAKI-1/RC)*, 77.90 (RXF 393/RC), *35.85 (TK-10/RC)*, 70.98 (UO-31/RC), 68.84 (PC-3/PC), 60.30 (DU-145/PC), *44.16 (MCF7/BC), 39.64 (MDA-MB-231/ATCC/BC)*, 61.22 (BT-549/BC), *56.31 (T-47D/BC)*
2.26	94.98	13.94 - 135.33	61.44 (CCRF-CEM/L), 61.17 (RPMI-8226/L), 77.24 (OVCAR-8/OV), *13.94 (CAKI-1/RC)*, 76.73 (MCF7/BC), 76.51 (T-47D/BC)
2.27	92.06	64.33 - 116.58	68.29 (NCI-H460/nscLC), 79.64 (HCT-116/ColC), 71.89 (HCT-15/ColC), *64.33 (HT29/ColC)*, 78.79 (IGROV1/OV), 77.47 (T-47D/BC)
2.28	94.93	64.98 - 112.15	79.08 (NCI-H522/nscLC), *64.98 (UO-31/RC)*
2.29	79.99	25.87 - 111.09	73.15 (CCRF-CEM/L), 67.69 (RPMI-8226/L), 71.69 (SR/L), 78.60 (A549/ATCC/nscLC), 66.25 (HOP-62/nscLC), 75.54 (NCI-H226/nscLC), 76.00 (NCI-H322M/nscLC), 74.39 (HCT-116/ColC), 70.34 (HCT-15/ColC), 60.89 (HT29/ColC), 63.47 (SF-268/CNSC), *25.87 (SF-539/CNSC)*, 63.98 (SNB-75/CNSC), 72.65 (LOX IMVI/M), 78.46 (MALME-3M/M), 78.66 (UACC-257/M), 62.67 (OVCAR-8/OV), 58.89 (SK-OV-3/OV), *53.47 (ACHN/RC)*, *48.54 (CAKI-1/RC)*, 70.27 (RXF 393/RC), 78.10 (SN12C/RC), 69.31 (TK-10/RC), 68.04 (UO-31/RC), 68.13 (MCF7/BC), 61.15 (MDA-MB-231/ATCC/BC), 75.66 (T-47D/BC)
2.31	94.09	61.19 - 122.77	61.19 (HOP-62/nscLC), 79.44 (SNB-75/CNSC), 70.29 (U251/CNSC), 78.34 (SK-OV-3/OV), 79.78 (786-0/RC), 78.05 (ACHN/RC), 73.79 (TK-10/RC), 84.42 (MDA-MB-231/ATCC/BC)

Table 1

Test compounds	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %*
2.32	82.81	40.47 - 109.65	76.08 (A549/ATCC/nscLC), *41.21 (HOP-62/nscLC*), 78.24 (NCI-H226/nscLC), *60.93 (SF-268/CNSC)*, *60.60 (SF-295/CNSC)*, *57.56 (SF-539/CNSC)*, 68.19 (SNB-19/CNSC), *57.96 (SNB-75/CNSC)*, *40.47 (U251/CNSC)*, 79.40 (MALME-3M/M), 60.62 (SK-MEL-2/M), 64.50 (IGROV1/OV), 70.44 (SK-OV-3/OV), *46.03 (786-0/RC)*, 73.72 (ACHN/RC), 66.76 (RXF 393/RC), *53.58 (TK-10/RC)*, 64.16 (MDA-MB-231/ATCC/BC), *49.76 (MDA-MB-468/BC)*
2.33	83.52	42.02 - 104.44	75.66 (CCRF-CEM/L), 67.19 (RPMI-8226/L), 78.06 (HOP-62/nscLC), 76.44 (NCI-H226/nscLC), 73.36 (NCI-H460/nscLC), 74.43 (HCT-116/ColC), 73.40 (HCT-15/ColC), *62.93 (HT29/ColC)*, 72.14 (OVCAR-4/OV), 78.23 (OVCAR-8/OV), *61.86 (SK-OV-3/OV)*, 78.84 (786-0/RC), *46.78 (ACHN/RC), 42.02 (CAKI-1/RC)*, 70.06 (RXF 393/RC), 79.63 (TK-10/RC), *67.93 (UO-31/RC)*, 70.28 (MCF7/BC), 77.81 (MDA-MB-231/ATCC/BC), *46.93 (T-47D/BC)*

Note. *L – leukemia, nscLC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV– ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer.

Table 2 – **Chemical structure and *in vitro* activities of the substituted isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*] quinazoline-3,10(14*bH*)-diones**

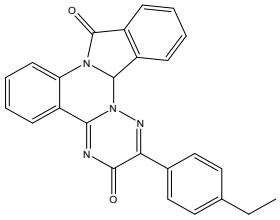
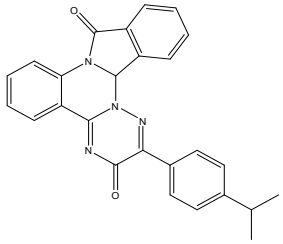
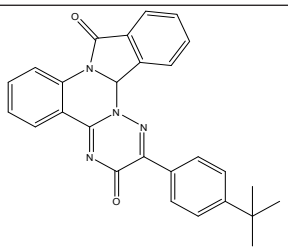
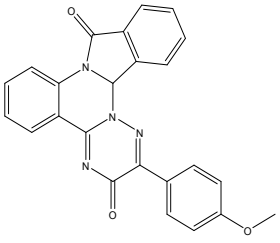
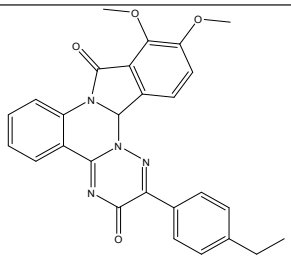
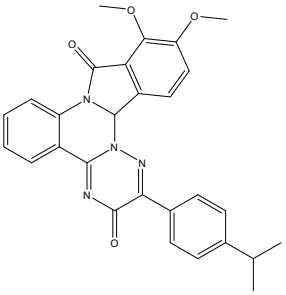
Comp.	Structure	Autodock scoring	Residual kinase activity (% at 33 μM*)	Residual kinase activity (% at 10 μM*)	IC_{50}, μM
2.3		-10.22	17	20	4.5
2.4		-10.64	16	46	n.e.**
2.5		-10.32	72	n.e.	n.e.
2.7		-8.75	14	36	3.6

Table 2

Comp.	Structure	Autodock scoring	Residual kinase activity (% at 33 μM*)	Residual kinase activity (% at 10 μM*)	IC50, μM
2.19		-8.98	16	36	4.7
2.20		-9.37	21	56	n.e.

Note. *Residual kinase activity is percent of kinase activity at inhibitor concentration 33 μM and 10 μM related to control with DMSO; **n.e. – not evaluated.

The chemical structure of active compounds can be the basis for the development of more active and selective inhibitors. With the aim of further optimization we have carefully studied the complexes of the compounds **2.3**, **2.7**, **2.19** with ATP-binding site of $FGFR_1$, obtained with molecular docking and predicted binding mode for inhibitors of this class.

Accordingly to the data of molecular modeling, all tested derivatives of 2-R_1-6-R_2-7-R_3-8-R_4-11-R_5-12-R_6-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14b*H*)-dione demonstrated similar binding mode with ATP-binding site of $FGFR_1$. As it can be seen from the Figure 3, 2,5-diazine heterocycle of compound **2.19** is located at the adenine-binding region of ATP-binding pocket and forms hydrophobic interactions with Val492, Ala512, Ala564, Leu630 and keto group on this heterocycle forms hydrogen bond with amide group of Ala564, which is in the hinge region of protein kinase. 4-ethyl-phenil substituent forms hydrophobic interactions with Tyr563 and Leu484. Keto group on 2,3-dyhidro-1H-isoindole-1-one and methoxy group in 7 position of this heterocycle form hydrogen bonds with conservative Lys514. All these interactions are important for ligand affinity to $FGFR_1$. The inhibitor also binds to the hydrophobic pockets I and II. These interactions are important for compounds selectivity [17].

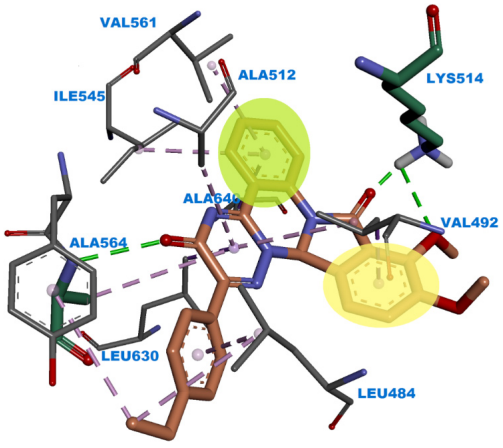


Figure 3. Binding mode of compound **2.19** in the active site of the FGFR1catalytic subunit. Hydrogen bonds are shown by the green dotted lines and hydrophobic interactions are presented by the magenta dotted lines. Hydrophobic pocket I is indicated by green circle, hydrophobic pocket II is labelled by yellow circle.

Accordingly to the analysis of structure-activity relationships of the studied compounds it was revealed that increasing of substituent hydrophobicity on the phenyl ring leads to significant decreasing of inhibitory activity toward $FGFR_1$ (isopropyl substituent in the structure of compounds **2.4** and **2.20**, isobutyl in the structure of compound **2.5**), because these substituents are directed in hydrophilic environment. The introduction of methoxy group into 3-dyhidro-1H-isoindole-1-one doesn't have significant impact on the compounds inhibitory activity against $FGFR_1$.

CONCLUSIONS. Interaction of 3-(2-amino-3-R_4-4-R_3-5-R_2-phenyl)-6-R_1-1,2,4-triazin-5(2H)-ones with 2-formylbenzoic or 6-formyl-2,3-dimethoxybenzoic (opianic) acids in glacial acetic acid leads

to formation of correspondent 2-R_1-6-R_2-7-R_3-8-R_4-11-R_5-12-R_6-3*H*-isoindolo[2,1-*a*][1,2,4]triazino[2,3-*c*]quinazoline-3,10(14b*H*)-diones with high yields. Mentioned transformation occurs as multistep process where substituted 2-(2-oxo-3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-c]quinazolin-6-yl) benzoic acids plays role of intermediate. Synthesized compounds reveal $FGFR_1$ inhibitory activity and significant anticancer action against certain cell lines. Docking study and SAR data revealed that that increasing of substituent hydrophobicity on the phenyl ring leads to significant decreasing of inhibitory activity toward $FGFR_1$.

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ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ[1]
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СИНТЕЗ, ПРОТИРАКОВА ТА $FGFR_1$ ІНГІБУЮЧА АКТИВНІСТЬ ПОХІДНИХ ІЗОІНДОЛО[2,1-*a*][1,2,4]ТРИАЗИНО[2,3-*c*]ХІНАЗОЛІНУ

Резюме

У представленій роботі описано синтез, протиракову та $FGFR_1$ інгібуючу активність раніше невідомих ізоіндоло[2,1-a][1,2,4]триазино[2,3-c]хіназолінів. Показано, що зазначені сполуки можна одержати в результаті взаємодії 3-(2-аміно-3-R_2-5-R_3-феніл)-6-R_1-1,2,4-триазин-5(2H)-онів з 2-формілбензойною кислотою або 6-форміл-2,3-диметоксибензойною (опіановою) кислотою в оцтовій кислоті. Встановлено, що відповідні 2-(2-оксо-3-R-6,7-дигідро-2H-[1,2,4]триазино[2,3-c]хіназолін-6-іл)бензойні кислоти (або їх диметоксивмісні аналоги) відіграють роль інтермедіатів реакції. Досліджено спектральні характеристики синтезованих сполук. Встановлено, що сигнал протону положення 8 реєструється в слабкому полі в результаті наявності водневого зв'язку між даним протоном та атомом оксигену положення 10. Дослідження протиракової дії дозволило ідентифікувати синтезовані сполуки як перспективні протипухлинні агенти. Також виявлено $FGFR_1$ інгібуючу дію синтезованих сполук та проведено відповідні докінгові дослідження.

КЛЮЧОВІ СЛОВА: **ізоіндоли, триазини, хіназоліни, протиракова активність, $FGFR_1$ інгібуюча активність, докінгові дослідження.**

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ЗАПОРОЖСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ[1]
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СИНТЕЗ, ПРОТИВОРАКОВАЯ И $FGFR_1$ ИНГИБИРУЮЩАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ ИЗОИНДОЛО[2,1-*a*][1,2,4]ТРИАЗИНО[2,3-*c*]ХИНАЗОЛИНА

Резюме

В представленной работе описаны синтез, противораковая и $FGFR_1$ ингибирующая активность ранее неизвестных изоиндоло[2,1-a][1,2,4]триазино[2,3-c]хиназолинов. Показано, что упомянутые соединения можно получить в результате взаимодействия 3-(2-амино-3-R_2-5-R_3-фенил)-6-R_1-1,2,4-триазин-5(2H)-онов с 2-формилбензойной кислотой или 6-формил-2,3-диметоксибензойной (опиановой) кислотой в уксусной кислоте. Установлено, что соответствующие 2-(2-оксо-3-R-6,7-дигидро-2H-[1,2,4] триазино[2,3-c]хиназолин-6-ил)бензойные кислоты (или их диметоксисодержащие аналоги) выступают в роли интермедиатов реакции. Исследованы спектральные характеристики синтезированных соединений. Установлено, что сигнал протона положения 8 регистрируется в слабом поле в результате наличия водородной связи между данным протоном и атомом кислорода положения 10. Исследование противоракового действия позволило идентифицировать синтезированные соединения как перспективные противоопухолевые агенты. Также выявлена $FGFR_1$ ингибирующая активность синтезированных соединений и проведены соответствующие докинговые исследования.

КЛЮЧЕВЫЕ СЛОВА: **изоиндолы, триазины, хиназолины, противораковая активность, $FGFR_1$ ингибирующая активность, докинговые исследования.**

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