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O.Yu. Voskoboynik

SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND ANTICANCER ACTIVITY OF 6-(HETEROCYCLYL-N-YLMETHYL)-3-R_1-9-R_2-2H-[1,2,4]TRIAZINO[2,3-C]-QUINAZOLIN-2-ONES

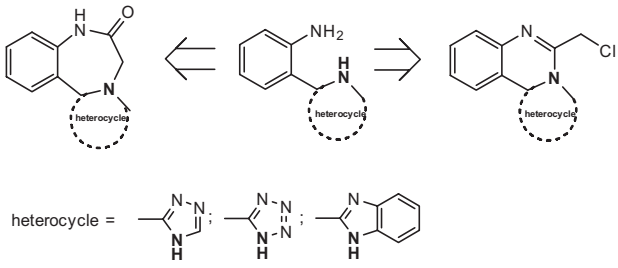
Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
The Department of organic and bioorganic chemistry

The effective and convenient methods for preparation of 6-(heterocyclyl-*N*-ylmethyl)-3-R_1-9-R_2-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones via interaction of secondary cyclic amines with 6-(chloromethyl)-3-R_1-9-R_2-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones were described in presented paper. It was shown, that synthesis of mentioned above intermediate has some features which are caused by possibility of alternative interaction, that yielded 3-R_1-10-R_2-benzo[*f*][1,2,4]triazino[2,3-*d*][1,4]diazepine-2,7(6*H*,8*H*)-diones. Compounds' individuality was proved by LC-MS, their structure - by ^{1}H NMR, ^{13}C NMR and mass spectral methods. The characteristic signals in ^{1}H NMR, ^{13}C NMR spectra were described, features of molecule fragmentation under electron impact were elucidated. Some of the obtained compounds were evaluated for anticancer action according to National Cancer Institute (USA) protocol. It was shown, that presence of fluorine and azepan moiety in molecule of studied compounds significantly improves as intensity, so spectrum of the antineoplastic action. The most intensive growth inhibitions were observed for cell lines of non-small lung cancer, renal cancer, breast cancer and leukemia. The perspectives of further investigations aimed to the creation of novel anticancer agents among described compounds were substantiated.

Keywords: triazines, quinazolines, synthesis, anticancer action, structure-activity relationships.

Introduction

It is known, that 1,5-binucleophiles are asked-for precursors for formation of various heterocyclic systems. Acyl halides of halogen containing carboxylic acids are of considerable interest among the great number of multicenter reagents, used for modification of mentioned compounds. For example, chloracetyl chloride and bromoacetyl bromide repeatedly were used in reactions with 2-heterylanilines for formation of novel condensed systems. We noted, that mentioned transformations occurred ambiguously, in spite of similarity of initial 2-heterylanilines and acyl halides (Scheme 1). Thus, according to [1] interaction between substituted 2-(1H-tetrazol-5-yl)anilines and bromoacetyl bromide yielded 5H-benzo[f]tetrazolo[1,5-d][1,4]diazepin-6(7H)-ones. In the same time, treating of substituted 2-(1H-1,2,4-triazol-5-yl)anilines and 2-(1H-benzo[d]imidazol-2-yl)aniline with chloracetyl chloride resulted corresponding condensed derivatives of quinazoline with chloromethyl moiety [2,3]. The last ones may be considered as promising objects of chemical modification aimed to create novel bioactive compounds. As we consider, introduction of saturated nitrogen containing heterocycle fragment is one of the most reasonable approaches for structural optimization of mentioned "scaffolds". Moreover recent publications described high value of this moiety as pharmacophore [4,5].



Scheme 1. Synthetic pathways, based on interaction of 2-herylanilines with chloracetyl chloride and bromoacetyl bromide

In our recent publication we have described the preparation of series of 3-(2-amino-3-R_2-4-R_3-5-R_4-)-6-R_1-1,2,4-triazin-5(2H)-one [6], and noted their potential as precursors for synthesis of

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[1,2,4]triazino[2,3-c]quinazolines. Considering the synthetic availability of mentioned above as 1,5-binucleophilic compounds, described features of interaction of 2-herylanilines with acyl halides of halogen containing carboxylic acids and practicability of combination of saturated nitrogen containing heterocycle fragment and planar [1,2,4]triazino[2,3-c]quinazoline system for creation of novel bioactive molecules, we aimed to elaborate synthetic protocols for 6-(heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones and study anticancer activity.

Results and discussion

Chemistry

As initial compounds 3-(2-amino-4-R_2-phenyl)-6-R_1-1,2,4-triazin-5(2H)-ones 1.1−1.4 were used. Experiments showed, that according to LC-MS data refluxing of initial compounds in glacial acetic acid with 1 eq. of chloracetyl chloride in presence of 1 eq. of sodium acetate during 6 hours yielded mixture of corresponding 6-(chloromethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones and 3-R_1-10-R_2-benzo[f][1,2,4]triazino[2,3-d][1,4]diazepine-2,7(6H,8H)-diones. To obtain target products, we decided to modify synthetic protocols by changing reagent ratio, as well as decreasing of temperature and duration of reaction. Thus, reaction of 1.1−1.4 and 1.6 fold excess of chloracetyl chloride at 60^{0}C during half an hour allowed to obtain compounds 2.1-2.4 with content yields. 6-(Heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (3.1−3.8) were synthesized via interaction of 2.1-2.4 with three fold excess of correspondent cyclic secondary amine in propan-2-ol during 6 hours. Potassium iodide was added as catalyst of alkylation process.

Individuality of compounds 2.1−2.4 and 3.1−3.8 was proved by LC-MS. Structure was established via set of characteristic signals in ^{1}H NMR, ^{13}C NMR and mass spectral data. In ^{1}H NMR − spectra of compounds 2.1−2.4 characteristic were signals of methylene fragment at 5.15−5.16 ppm, benzene fragment of quinazoline system and substituent in position 3 were observed.

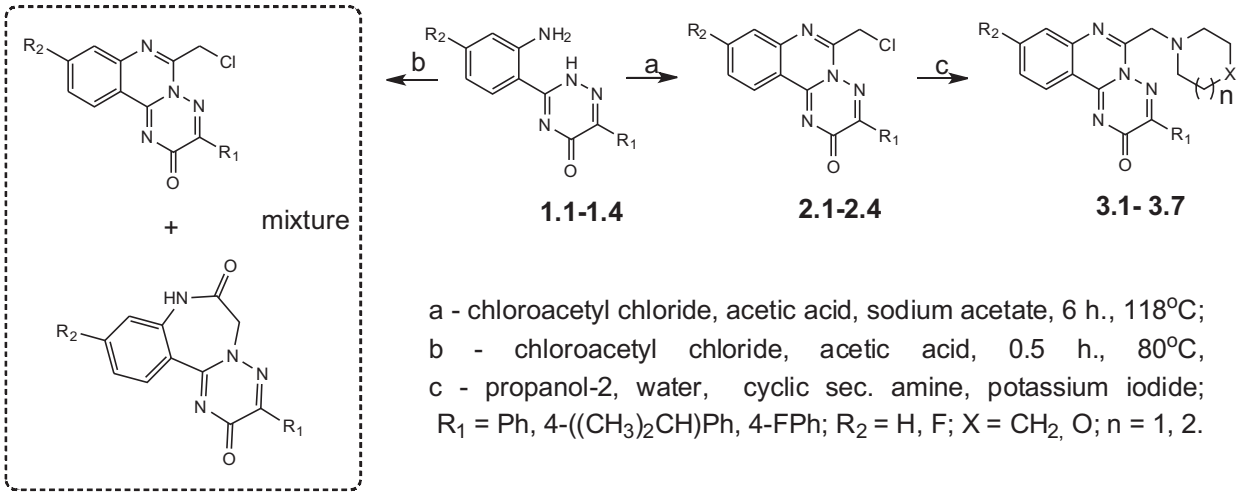
^{1}H and ^{13}C NMR spectral data of compounds 3.1−3.8 totally correspond to their structure. Thus, signals of methylene carbon atom were observed at 4.01−4.30, signals of moiety which contain saturated nitrogen were registered in aliphatic part of the spectrum, as series of two broad singlets of morpholine fragment at 3.62−3.63 ppm and 2.73−2.74 ppm for compounds 3.2, 3.5, 3.8, three broad singlets of piperidine cycle at 2.68−2.73 ppm, 1.57−1.59 ppm and 1.46−1.48 ppm for compounds 3.1, 3.3, 3.6 and multiplets of azepane fragment at 2.95−3.00 ppm and 1.37−1.92 ppm (3.4, 3.7).

Proposed structures were additionally proved by ^{13}C NMR spectral data. Thus, in spectrum of compound 3.2 characteristic signals of C2 carbon atom were observed at 158.74 ppm, methylene carbon at 58.53 ppm, morpholine fragment at 53.18 ppm and 66.08 ppm.

Compound 3.2 is characterized by absence of molecular ion signal in mass-spectra (EI). The most intensive peak (m/z 186) is caused by the consecutive elimination of morpholine fragment and cleavage of the C2-N3 and N4-N5 bonds. Presented direction of fragmentation can be additionally proved by the low intensive ion with m/z 289, which is formed via elimination of morpholine moiety.

Anticancer activity

Compounds 3.2 and 3.7 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



Scheme 2. Synthesis of 6-(chloromethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones and 6-(heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones

according to the US NCI protocol, which was described elsewhere [7]. 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.

Experimental data showed, that compound 3.2 was almost inactive corresponding to studied cell lines. Range of growth was 77.49–123.14%, mean growth – 101.16%. The most sensitive line was renal cancer cell line UO-31 (growth inhibition level 22.51%), growth of all other cell lines was similar to untreated control cells. Introduction of fluorine in position 9 and changing of morpholine moiety by azepane significantly improve as intensity, so spectrum of the action. Thus, for compound 3.7 range of growth was 41.58–117.83%, mean growth – 84.65%. The most intensive growth inhibition were observed for lines of non-small cell lung cancer (NCI-H460 - 58.42%, A549/ATCC – 47.91%) and renal cancer (ACHN – 40.27%, UO-31 – 44.4%). Besides, the last compound was active towards MCF7-line of breast cancer (growth inhibition – 51.60%) and SR-line of leukemia (growth inhibition – 45.81%). As we consider, in spite of few examples, obtained data allow to affirm, that described compounds are promising objects of investigations aimed to the creation of novel anticancer agents. Our confidence is based on the fact of significant increasing of anticancer action after minor modification of structure.

Conclusion

6-(Heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones can be obtained via interaction of 3-R-6-(chloromethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones with correspondent cyclic secondary amines. Some of synthesized compounds exhibit anticancer action against leukemia, non-small cell lung cancer, renal cancer, breast cancer cell lines and may be used as objects of structure optimisation aimed to the creation of the novel antineoplastic agents.

Experimental part

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).

Percentage of in vitro tumor cell lines growth at 10 μM for synthesized compounds (3.2, 3.7)

Compd.	Mean growth, %	Range of growth, %	Cell line growth, %*
3.2	101.16	77.49–123.14	91.82 (K-562/L), 98.75 (RPMI-8226/L), 92.81 (A549/ATCC/nscLC), 92.08 (HOP-62/nscLC), 91.58 (HOP-92/nscLC), 97.18 (NCI-H226/nscLC), 98.35 (NCI-H23/nscLC), 89.62 (NCI-H322M/nscLC), 92.97 (NCI-H522/nscLC), 106.89 (COLO 205/ColC), 99.32 (HCC-2998/ColC), 99.76 (HT29/ColC), 99.77 (KM12/ColC), 92.60 (SF-268/CNSC), 94.27 (SNB-75/CNSC), 94.19 (U251/CNSC), 93.34 (SK-MEL-5/M), 98.24 (UACC-257/M), 98.28 (OVCAR-3/OV), 95.89 (SK-OV-3/OV), 96.52 (CAKI-1/RC), 94.28 (TK-10/RC), 77.49 (UO-31/RC), 84.36 (PC-3/PC), 95.84 (MCF7/BC), 96.58 (BT-549/BC)
3.7	84.65	41.58–117.83	86.44 (CCRF-CEM/L), 78.92 (HL-60(TB)/L), 86.00 (K-562/L), 43.96 (MOLT-4/L), 90.81 (RPMI-8226/L), 54.19 (SR/L), 52.09 (A549/ATCC/nscLC), 92.33 (HOP-62/nscLC), 78.92 (HOP-92/nscLC), 75.11 (NCI-H226/nscLC), 95.91 (NCI-H322M/nscLC), 41.58 (NCI-H460/nscLC), 78.96 (NCI-H522/nscLC), 76.57 (COLO 205/ColC), 73.56 (HCT-116/ColC), 90.60 (HCT-15/ColC), 84.27 (HT29/ColC), 65.82 (SW-620/ColC), 98.99 (SF-268/CNSC), 78.26 (SF-295/CNSC), 84.83 (SNB-19/CNSC), 74.39 (SNB-75/CNSC), 74.79 (U251/CNSC), 71.62 (LOX IMVI/M), 79.00 (SK-MEL-5/M), 94.71 (UACC-257/M), 85.10 (UACC-62/M), 84.56 (IGROV1/OV), 87.68 (OVCAR-4/OV), 89.59 (OVCAR-8/OV), 94.64 (NCI/ADR-RES/OV), 87.41 (786-0/RC), 59.73 (ACHN/RC), 65.49 (CAKI-1/RC), 89.45 (RXF 393/RC), 74.91 (SN12C/RC), 95.87 (TK-10/RC), 55.54 (UO-31/RC), 89.29 (PC-3/PC), 75.10 (DU-145/PC), 48.40 (MCF7/BC), 89.34 (BT-549/BC), 78.00 (T-47D/BC), 88.79 (MDA-MB-468/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.

Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000−600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz): were recorded on a Varian-Mercury 400 (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 1.1−1.4 were obtained according to the described synthetic protocols [6].

General method for the preparation of 6-(chloromethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (2.1−2.4). The 10 mmol of corresponded 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-ones (1.1−1.4) was suspended in 30 ml of acetic acid and 16 mmol of chloracetyl chloride was added. The formed mixture was stirred at 60^{0}C during 30 min. The mixture was cooled, poured in water. Formed precipitate was filtered off, dried and crystallized from acetone.

6-(Chloromethyl)-3-phenyl-2H-[1,2,4]-triazino[2,3-c]quinazolin-2-one (2.1) Yield 89.07%; M.p. 218−220^{0}C; ^1H NMR δ 8.64 (d, J=7.9 Hz, 1H, H-11), 8.35 (d, J=7.3 Hz, 2H, 3 Ph H-2,6), 8.04 (t, J=7.5 Hz, H-9), 7.94 (d, J=8.0 Hz, 1H, H-8), 7.82 (t, J=7.4 Hz, 1H, H-10), 7.69−7.43 (m, 3H, 3 Ph H-3,4,5), 5.19 (s, 2H, CH_2); LC-MS m/z=323.0 [M+1]; Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.31; H, 3.49; N, 17.41; Found: C, 63.35; H, 3.53; N, 17.45.

6-(Chloromethyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (2.2) Yield 83.20%; M.p. 250−252^{0}C; ^1H NMR 8.66 (d, 1H, J=7.7 Hz, H-11), 8.30 (d, 2H, J=8.2 Hz, H-2', H-6'); 8.02 (t, 1H, J=7.2 Hz, H-9), 7.92 (d, 1H, J=7.0 Hz, H-8), 7.81 (t, 1H, J=7.5 Hz, H-10), 7.39 (d, 2H, J=8.2 Hz, 2H, H-3', H-5'), 5.16 (s, 2H, CH_2Cl), 3.04−2.97 (m, 1H, $CH(CH_3)_2$), 1.33 (d, 6H, J=6.8 Hz, $CH(CH_3)_2$); LC-MS m/z=365 [M+1]; Anal. calcd. for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36; Found: C, 65.87; H, 4.71, N, 15.38.

6-(Chloromethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (2.3) Yield 83.20%; M.p. 250−252^{0}C; ^1H NMR 8.66 (d, 1H, J=7.7 Hz, H-11), 7.81 (t, 1H, J=7.4 Hz, H-10), 8.48 (dd, 2H, J=8.7,5.6 Hz, H-2', H-6'), 8.03 (t, 1H, J=7.1 Hz, H-9), 7.93 (d, 1H, J=8.0 Hz, H-8), 7.29 (t, 1H, J=8.7 Hz, H-3', H-5'), 5.18 (s, 2H, CH_2Cl); EI-MS m/z (I rel, %)=221 (30.1). 220 (11.00). 219 (100.0), 185 (6.9), 184 (11.4), 177 (5.1), 143 (5.3), 142 (9.9), 122 (5.4), 121 (62.0), 116 (10.8), 115 (5.1), 107 (13.8), 102 (30.1), 95 (9.0), 94 (29.1), 90 (9.7), 89 (9.2), 88 (5.2), 81 (6.2), 76 (17.6), 75 (18.2), 64 (5.5), 63 (7.7), 57 (10.3), 56 (40.7), 51 (12.7), 50 (8.3), 49 (14.7); LC-MS m/z=341 [M+1]; Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44; Found: C, 59.98; H, 3.01, N, 16.48.

6-(Chloromethyl)-9-fluoro-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (2.4) Yield 86.80%; M.p.=243−245^{0}C; ^1H NMR d 9.15−8.53 (m, 1H, H-11), 8.34 (d, J=7.4 Hz, 2H, 3 Ph H-2,6), 7.68−7.47 (m, 5H, H-8,10; 3 Ph H-3,4,5), 5.15 (s, 2H, CH_2); LC-MS m/z=341.0 (M+1); Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44; Found C, 59.98; H, 3.03; N, 16.51

General method for the preparation of 6-(heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]-triazino[2,3-c]quinazolin-2-ones (3.1−3.8). The 5 mmol of corresponded 6-(chloromethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (2.1−2.4) was suspended in 20 ml of propan-2-ol, then 15 mmol. of corresponding cyclic secondary amine, 2 drops of water and 0.02 g. of potassium iodide were added. The formed mixture was refluxed during 6 h. and cooled. The formed solid was filtered and dried.

3-Phenyl-6-(piperidin-1-ylmethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.1) Yield 34.73%; M.p.=227−229^{0}C; ^1H NMR d 8.64 (d, J=7.8 Hz, 1H, H-11), 8.29 (d, J=7.0 Hz, 2H, 3 Ph H-2,6), 8.00 (t, J=7.7 Hz, 1H, H-9), 7.89 (d, J=7.7 Hz, 1H, H-8), 7.76 (t, J=7.1 Hz, 1H, H-10), 7.55 (m, 3H, 3 Ph H-3,4,5), 4.07 (s, 2H, CH_2), 2.73 (bs, 4H, piperidin H-2,2',6,6'), 1.59 (bs, 4H, piperidin H-3,3',5,5'), 1.48 (bs, 2H, piperidin H-4,4'); LC-MS m/z=372.2 (M+1); Anal. calcd. for $C_{22}H_{21}N_5O$: C, 71.14; H, 5.70; N, 18.85; Found: C, 71.16; H, 5.73; N, 18.89.

6-(Morpholinomethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.2) Yield 46.64%; M.p.=216−218^{0}C; ^1H NMR δ 8.63 (d, J=7.6 Hz, 1H, H-11), 8.27 (d, J=6.8 Hz, 2H, 3 Ph H-2,6), 8.00 (t, J=7.6 Hz, 1H, H-9), 7.89 (d, J=7.8 Hz, 1H, H-8), 7.77 (t, J=7.2 Hz, 1H, H-10), 7.63−7.42 (m, 3H, 3 Ph H-3,4,5), 4.12 (s, 2H, CH_2), 3.63 (bs, 4H, morpholine H-2,2',6,6'), 2.75 (bs, 4H, 3,3',5,5'). ^{13}C NMR (101 MHz, DMSO) δ 158.74, 150.89, 149.74, 149.12, 142.90, 134.93, 131.89, 130.86, 129.18, 128.48, 127.92, 127.50, 125.39, 119.41, 66.08, 58.53, 53.18; MS-EI, m/z ($I\%_{rel.}$) 289 (2.5), 187 (11.9), 186 (100), 146 (11.4), 144 (15.2), 143 (13.6), 129 (20.1), 117 (9.4), 116 (7.9), 104 (7.8), 103 (68.9), 102 (52.3), 101 (5.8), 100 (36.5), 98 (7.2), 90 (21.1), 89 (12.6), 86 (18.7), 77 (25.2), 76 (64.7), 75 (10.6), 70 (13.7), 69 (7.1), 65

(5.4), 64 (9.2), 63 (16.3), 56 (63.7), 54 (5.5), 50 (5), 43 (5.2), 42 (44.3), 41 (29.2); LC-MS m/z=374.2 (M+1); Anal. calcd. for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76; Found: C, 67.61; H, 5.16; N, 18.81.

3-(4-Isopropylphenyl)-6-(piperidin-1-ylmethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.3) Yield 73.86%; M.p.=201−204^{0}C; ^{1}H NMR δ 8.62 (d, J=7.8 Hz, 1H, H-11), 8.20 (d, J=8.0 Hz, 2H, 3 Ph H-2,6), 7.95 (t, J=7.3 Hz, 1H, H-9), 7.85 (d, J=8.0 Hz, 1H, H-8), 7.72 (t, J=7.4 Hz, 1H, H-10), 7.34 (d, J=8.0 Hz, 2H, 3 Ph H-3,5), 4.01 (s, 2H, CH_2), 2.99 (m, 1H, $\underline{CH}(CH_3)_2$), 2.69 (bs, 4H, piperidin H-2,2',6,6'), 1.59 (s, 4H piperidin H-3,3',5,5'), 1.46 (s, 2H, 4H piperidin H-4,4'), 1.32 (d, J=6.8 Hz, 6H, $CH(C\underline{H}_3)_2$); LC-MS m/z=414.2 (M+1); Anal. calcd. for $C_{24}H_{25}N_5O_2$: C, 72.61; H, 6.58; N, 16.94; Found C, 72.67; H, 6.63; N, 16.99.

6-(Azepan-1-ylmethyl)-3-(4-isopropylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.4) Yield 38.30%; M.p.=172−175^{0}C; ^{1}H NMR δ 8.63 (d, J=7.7 Hz, 1H, H-11), 8.22 (d, J=7.1 Hz, 1H, 3 Ph H-2,6), 7.96 (t, J=7.5 Hz, 1H, H-9), 7.85 (d, J=7.5 Hz, 1H, H-8), 7.73 (t, 1H, J=7.5 Hz, 1H, H-10), 7.34 (d, J=7.3 Hz, 1H, 3 Ph H-3,5), 4.30 (s, 2H, CH_2), 3.00 (m, 1H, $\underline{CH}(CH_3)_2$, azepan 2,2',7,7'), 1.92−1.47 (m, 8H, azepane 3,3',4,4', 5,5',6,6'), 1.32 (d, J=6.1 Hz, 1H, $CH(\underline{CH_3)_2}$); LC-MS m/z=428.2 (M+1); Anal. calcd. for $C_{26}H_{29}N_5O$: C, 73.04; H, 6.84; N, 16.38; Found C, 73.09; H, 6.89; N, 16.43.

3-(4-Isopropylphenyl)-6-(morpholinomethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.5) Yield 75.27%; M.p.=225−227^{0}C; ^{1}H NMR δ 8.63 (d, J=7.8 Hz, 1H,), 8.20 (d, J=7.8 Hz, 2H, 3 Ph H-2,6), 7.96 (t, J=7.4 Hz, 1H, H-9), 7.86 (d, J==7.9 Hz, 1H, H-8), 7.73 (t, J=7.4 Hz, 1H, H-10), 7.35 (d, J=7.8 Hz, 2H, 3 Ph H-3,5), 4.10 (s, 2H, CH_2), 3.63 (s, 4H, morpholine H-2,2',6,6'), 3.00 (s, 1H, $\underline{CH}(CH_3)_2$), 2.74 (s, 4H, morpholine H-3,3',5,5'), 1.32 (d, J=6.7 Hz, 1H, $CH(\underline{CH_3)_2}$); LC-MS m/z=416.2 (M+1); Anal. calcd. for $C_{24}H_{25}N_5O_2$: C, 69.38; H, 6.07; N, 16.86; Found: C, 69.44; H, 6.13; N, 16.92.

3-(4-Fluorophenyl)-6-(piperidin-1-ylmethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.6) Yield 61.67%; M.p.=229−232^{0}C; ^{1}H NMR (r 8.64 (d, J=7.9 Hz, 1H, H-11), 8.42−8.32 (m, 2H, 3 Ph H-2,6), 7.97 (t, J=7.4 Hz, 1H, H-9), 7.86 (d, J==7.9 Hz, 1H, H-8), 7.74 (t, J=7.3 Hz, 1H, H-10), 7.25 (t, J=8.6 Hz, 2H, 3 Ph H-3,5), 4.02 (s, 2H, CH_2), 2.68 (bs, 2H, piperidin H-2,2',6,6'), 1.57 (bs, 4H, piperidin H-3,3',5,5'), 1.46 (bs, 2H, piperidin H-4,4); LC-MS m/z=372.2 (M+1); Anal. calcd. for $C_{22}H_{20}FN_5O$: C, 67.85; H, 5.18; F, 4.88; N, 17.98; Found: C, 67.89; H, 5.21; F, 4.92; N, 18.03.

6-(Azepan-1-ylmethyl)-3-(4-fluorophenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.7) Yield 72.79%; M.p.=190−193^{0o}C; ^{1}H NMR δ 8.63 (d, J=7.9 Hz, 1H, H-11), 8.49−8.34 (m, 2H, 3 Ph H-2,6), 7.96 (t, J=7.4 Hz, 1H, H-9), 7.86 (d, J==7.9 Hz, 1H, H-8), 7.73 (t, J=7.3 Hz, 1H, H-10), 7.24 (t, J=8.3 Hz, 2H, 3 Ph H-3,5), 4.30 (s, 2H, CH_2), 2.95 b(s, 4H, azepan 2,2',7,7'), 1.83−1.37 (m, 8H, azepane 3,3',4,4',5,5',6,6'); LC-MS m/z=404.1 (M+1); Anal. calcd. for $C_{23}H_{22}FN_5O$: C 68.47; H, 5.50; F, 4.71; N, 17.36; Found:C 68.49; H, 5.7 F, 4.76; N, 17.42.

9-Fluoro-6-(morpholinomethyl)-3-phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (3.8) Yield 44.05%; M.p.=237−238^{0}C; ^{1}H NMR δ 8.73−8.67 (m, 1H, H-11), 8.25 (d, J=7.2 Hz, 2H, 3 Ph H-2,6), 7.77−7.31 (m,5H, H-8,10,3 Ph H-3,4,5), 4.10 (s, 2H, CH_2), 3.62 (bs, 4H, morpholine H-2,2',6,6'), 2.73 (bs, 4H, morpholine H-3,3',4,4'); LC-MS m/z=392.0 (M+1); Anal. calcd. for $C_{21}H_{18}FN_5O_2$: C, 64.44; H, 4.64; N, 17.89; Found: C, 64.50; H, 4.71; N, 17.95.

Cytotoxic activity against malignant human tumor cells

Primary anticancer assay was performed at 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [7]. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

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SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND ANTICANCER ACTIVITY OF 6-(HETEROCYCLYL-N-YLMETHYL)-3-R_1-9-R_2-2*H*-[1,2,4]TRIAZINO[2,3-C]QUINAZOLIN-2-ONES

O.Yu. Voskoboynik

Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine

The effective and convenient methods for preparation of 6-(heterocyclyl-N-ylmethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones via the interaction of secondary cyclic amines with 6-(chloromethyl)-3-R_1-9-R_2-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones are described in the paper. The synthesis of the intermediate mentioned above has some features associated with the alternative possibility of the formation of 3-R_1-10-R_2-benzo[f]-[1,2,4]triazino[2,3-d][1,4]diazepine-2,7(6H,8H)-diones. The individuality of the synthesized compounds has been proved by liquid chromatography-mass spectrometry; their structure has been confirmed by ^{1}H NMR, ^{13}C NMR and mass spectrometry. The characteristic signals in ^{1}H NMR and ^{13}C NMR spectra are determined. The features of molecule fragmentation under the electron impact are elucidated. Some of the obtained compounds are evaluated for anticancer action according to National Cancer Institute (USA) protocol. The presence of fluorine or azepan fragment in the molecule of the compounds under study appreciably improves both the intensity and the spectrum of the cytotoxic action. The most intensive inhibition of the cancerous cells enlargement is observed for the lines of non-small cell lung cancer, renal cancer, breast cancer and leukemia. The findings support the perspectives of the further investigations aimed to searching novel anticancer agents among the compounds under consideration.

Keywords: triazines; quinazolines; synthesis; anticancer action; structure-activity relationships.

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