2-(Dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines: synthesis and anticancer activity

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Aim. Synthesis of a series of 2-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines and evaluation in vitro of their anticancer activity against a panel of 60 cell lines derived from nine cancer types, namely leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer. Methods. Organic synthesis; biological tests; spectral methods; statistical methods. Results. In vitro screening of the anticancer activity showed that 5 of 26 tested compounds can effectively inhibit the growth of certain cancer cell lines. Conclusions. New type of N-(2,2-dichloro-1-cyanoethenyl)carboxamides heterocyclization with 1H-pyrazol-5-amines led to the formation of 2-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines. Some of these compounds inhibit growth of certain cancer cell lines.

Keywords: in vitro screening, anticancer activity, heterocyclization, 1H-pyrazol-5-amines, pyrazolo[1,5-a][1,3,5]triazines, 2-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines.

Introduction

Organic synthesis plays a vital role in drug discovery, and modern synthetic methods focus on increasing the efficiency of preparing small drug-like molecules which include new drugs and drug candidates and reagents used to explore biological processes [1]. Pyrazolo[1,5-a][1,3,5]triazines were reported to behave as purine bioisosteres of various cycline-dep-
colorectal cancer cell lines [8]. 1H-Pyrazol-5-amines (or 5-aminopyrazoles) comprise a class of flexible nitrogen-containing aromatic heterocycles used as privileged organic tools for the construction of diverse fused heterocyclic scaffolds with versatile functionalities [9].

N-(2,2-Dichloro-1-cyanoethenyl)carboxamides are versatile highly reactive electrophilic reagents that are increasingly used in the organic synthesis, in particular in the synthesis of new types of heterocyclic compounds. Pioneering work on the development of cyclo-condensation reactions of N-(2,2-Dichloro-1-cyanoethenyl)carboxamides with N-nucleophiles originated in the late 1970’s by two research groups of Matsumura and Drach; it was found that these cyclocondensations with various N-nucleophiles constitute a facile method for the synthesis of novel 5-amino-4-cyanooxazoles [10–14], imidazole [13, 16], pyrazolo[1,5-a]pyrimidine [17], 7,8-dihydro-imidazo[1,2-c][1,3]oxazolo[4,5-e]pyrimidine [18], 7,8-dihydroimidazo[1,2-c][1,3]thiazolo[4,5-e]pyrimidine [19], 4,5,7,8-tetrahydro-imidazo[1,2-c][1,3]thiazolo[4,5-e][1,3,2]diazaphosphinine [20], and 1,2-dihydro-2λ5-[1,3]oxazolo[5,4-d][1,3,2]diazaphosphinine [21] derivatives. These achievements inspired us to develop an efficient method for the synthesis of new compounds with pyrazolo[1,5-a][1,3,5]triazine moiety. The current study was aimed at the synthesis of new 2-(dichloromethyl) pyrazolo[1,5-a][1,3,5]triazines starting from 1H-pyrazol-5-amines with N-(2,2-dichloro-1-cyanoethenyl)carboxamides, and in vitro evaluation of the obtained heterocycles’ anticancer activity against a panel of 60 cell lines derived from nine cancer types.

Materials and Methods

Chemistry

A series of new pyrazolo[1,5-a][1,3,5]triazine derivatives 3 for in vitro screening for anticancer activity was synthesized starting with N-(2,2-dichloro-1-cyanoethenyl)carboxamides 1 with 1H-pyrazol-5-amines 2 (Scheme 1).

All reagents and solvents used in synthetic procedures were purchased from Aldrich and used as received. The reaction progress was monitored by the TLC method on Silica gel 60 F254 Merck. 1H (400 MHz) and 13C (100 MHz)

![Scheme 1](image-url)

R1, R2 = Me (a), Ph (b), 4-MeC6H4 (c), 4-MeOC6H4 (d), 4-FC6H4 (e), 4-ClC6H4 (f), t-Bu (g)
NMR spectra of obtained products were recorded at Varian Unityplus 400 spectrometer in DMSO-$d_6$ solution with TMS as the internal standard. IR spectra were recorded on a Vertex 70 spectrometer from KBr pellets. Melting points were measured on a Fisher-Johns instrument.

Chromatomass spectra were recorded on an Agilent 1100 Series high performance liquid chromatograph equipped with a diode matrix with an Agilent LC/MS mass selective detector allowing a fast switching of the positive/negative ionization modes (chemical ionization).

Elemental analyses were performed at the Analytical Laboratory of the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine, their results were found to be in good agreement (±0.4 %) with the calculated values.

**General procedures of 2-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines 3 synthesis.**

5-aminopyrazole (0.01 mol) and Et$_3$N (1.39 ml, 0.01 mol) were added to a solution of 2-acyl-amino-3,3-dichloroacrylonitrile 1 (0.01 mol) in 10 ml of THF. The mixture was stirred at room temperature for 24 h, and then heated at 55–60 °C for 2 h. After solvent evaporation the residue was triturated with water to give a crude product which was dried and recrystallized to obtain yellow or brownish crystals.

When assigning signals in the $^1$H and $^{13}$C NMR spectra, the atoms of the aryl substituents at position 4 of the heterocycle are designated as H’ and C’, respectively, the atoms of the aryl substituents in position 7 are designated as H” and C”.

2-Dichloromethyl-4,7-dimethylpyrazolo[1,5-a][1,3,5]triazine (3aa). Mp 108–110 °C (H$_2$O). IR, υ, cm$^{-1}$: 3101, 3021, 3003, 2929, 1681, 1603, 1526, 1358, 1254, 843, 786, 743, 667. $^1$H NMR, δ, ppm (J, Hz): 2.49 (3H, s, Me-7), 2.91 (3H, s, Me-4), 6.69 (1H, s, H-8), 7.26 (1H, s, CHCl$_2$). $^{13}$C NMR, δ, ppm: 14.5 (Me-7), 19.2 (Me-4), 70.9 (CHCl$_2$), 97.6 (C-8), 147.5, 157.2, 158.0, 158.8. MS, m/z 231.1 [M+H]$^+$.  

2-Dichloromethyl-4-methyl-7-phenylpyrazolo[1,5-a][1,3,5]triazine (3ab). Mp 194–196 °C (MeCN). IR, υ, cm$^{-1}$: 3012, 1606, 1529, 1457, 1253, 836, 787, 764, 736, 688, 655. $^1$H NMR, δ, ppm (J, Hz): 3.01 (3H, s, Me-4), 7.23 (1H, s, H-8 or CHCl$_2$), 7.34 (1H, s, H-8 or CHCl$_2$), 7.50–7.54 (3H, m, H-3′′–5′′), 8.09 (2H, d, J = 5.0, H-2′′,6′′). $^{13}$C NMR, δ, ppm: 19.2 (Me-4), 70.9 (CHCl$_2$), 95.0 (C-8), 126.7, 129.0, 131.0, 131.3, 148.3, 157.5, 157.9, 159.4. MS, m/z 293.0 [M+H]$^+$.  

2-Dichloromethyl-7-(4-fluorophenyl)-4-methylpyrazolo[1,5-a][1,3,5]triazine (3ac). Mp 172–174 °C (EtOH). IR, υ, cm$^{-1}$: 3001, 1600, 1523, 1449, 1255, 843, 775, 657. $^1$H NMR, δ, ppm (J, Hz): 2.38 (3H, s, Me-4′′), 2.98 (3H, s, Me-4), 7.34–7.35 (3H, m, H-8 or CHCl$_2$, H-3′′,5′′), 7.39 (1H, s, H-8 or CHCl$_2$), 8.00 (2H, d, J = 8.0, H-2′′,6′′). $^{13}$C NMR, δ, ppm: 19.2 (Me-4), 21.0 (Me-4′′), 70.9 (CHCl$_2$), 94.7 (C-8), 126.6, 128.5, 129.6, 139.8, 148.3, 157.4, 158.0, 159.3. MS, m/z 307.0 [M+H]$^+$.  

2-Dichloromethyl-7-(4-fluorophenyl)-4-methylpyrazolo[1,5-a][1,3,5]triazine (3ac). Mp
175–177 °C (MeCN). IR, υ, cm\(^{-1}\): 3009, 1612, 1521, 1449, 1234, 1094, 844, 779, 739, 660.

\(^1\)H NMR, δ, ppm: 2.98 (3H, s, Me-4), 7.33–7.42 (4H, m, CHCl\(_2\), H-8,3′,5′,5′′), 8.13–8.17 (2H, m, H-2′,6′). \(^1\)C NMR, δ, ppm (J, Hz): 19.7 (Me-4), 71.3 (CHCl\(_2\)), 95.4 (C-8), 116.5 (d, J = 21.9, C-3′,5′), 128.4 (d, J = 3.0, C-1′′), 129.5 (d, J = 8.5, C-2′,6′), 148.9, 157.4, 158.1, 159.9, 163.7 (d, J = 247.3, C-4′). MS, m/z 311.0 [M+H]⁺.

2-Dichloromethyl-7-methyl-4-phenylpyrazolo[1,5-a][1,3,5]triazine (3ba). Mp 120–122 °C (EtOH). IR, υ, cm\(^{-1}\): 3008, 1600, 1528, 1485, 1236, 1183, 839, 783, 735, 686, 669, 643, 530. \(^1\)H NMR, δ, ppm (J, Hz): 2.98 (3H, s, Me-4), 7.33–7.42 (4H, m, CHCl\(_2\), H-8,3′,5′,5′′), 8.13–8.17 (2H, m, H-2′,6′). \(^1\)C NMR, δ, ppm (J, Hz): 19.7 (Me-4), 71.3 (CHCl\(_2\)), 95.4 (C-8), 116.5 (d, J = 21.9, C-3′,5′), 128.4 (d, J = 3.0, C-1′′), 129.5 (d, J = 8.5, C-2′,6′), 148.9, 157.4, 158.1, 159.9, 163.7 (d, J = 247.3, C-4′). MS, m/z 311.0 [M+H]⁺.

2-Dichloromethyl-7-(4-fluorophenyl)-4-phenylpyrazolo[1,5-a][1,3,5]triazine (3be). Mp 163–165 °C (MeCN+DMF, 2:1). IR, υ, cm\(^{-1}\): 3085, 2924, 1607, 1589, 1477, 1451, 1226, 840, 795, 781, 663. \(^1\)H NMR, δ, ppm (J, Hz): 2.98 (3H, s, Me-4), 7.33–7.42 (4H, m, CHCl\(_2\), H-8,3′,5′,5′′), 8.13–8.17 (2H, m, H-2′,6′). \(^1\)C NMR, δ, ppm (J, Hz): 19.7 (Me-4), 71.3 (CHCl\(_2\)), 95.4 (C-8), 116.5 (d, J = 21.9, C-3′,5′), 128.4 (d, J = 3.0, C-1′′), 129.5 (d, J = 8.5, C-2′,6′), 148.9, 157.4, 158.1, 159.9, 163.7 (d, J = 247.3, C-4′). MS, m/z 311.0 [M+H]⁺.
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844, 787, 746, 664. 1H NMR, δ, ppm (J, Hz): 2.39 (3H, s, Me-4'), 3.94 (3H, s, MeO-4'), 7.26 (2H, d, J = 8.8, H-3',5'), 7.34–7.39 (4H, m, H-8, CHCl2, H-3'',5''), 8.01 (2H, d, J = 8.0, H-2'',6''), 8.98 (2H, d, J = 8.8, H-2',6'). 13C NMR, δ, ppm: 21.5 (Me-4'), 56.3 (MeO-4'), 71.6 (CHCl2), 94.7 (C-8), 114.7, 121.8, 127.2, 129.0, 130.1, 134.2, 140.4, 151.3, 153.8, 158.0, 158.8, 164.2. MS, m/z 399.0 [M+H]+.

2-Dichloromethyl-7-(4-fluorophenyl)-4-(4-methoxyphenyl)pyrazolo[1,5-a][1,3,5]triazine (3de). Mp 175–177 °C (MeCN+DMF, 2:1). IR, v, cm−1: 3009, 2923, 1608, 1505, 1481, 1448, 1260, 1228, 1152, 838, 796, 742, 663. 1H NMR, δ, ppm (J, Hz): 3.94 (3H, s, MeO-4'), 7.26 (2H, d, J = 9.0, H-3',5'), 7.38–7.42 (3H, m, H-8 or CHCl2, H-3'',5''), 7.47 (1H, s, H-8 or CHCl2), 8.20–8.22 (2H, m, H-2'',6''), 8.97 (2H, d, J = 9.0, H-2',6'). 13C NMR, δ, ppm (J, Hz): 56.2 (MeO-4'), 71.6 (CHCl2), 95.0 (C-8), 114.7, 116.5 (d, J = 21.4, C-3',5''), 121.7, 128.4 (d, J = 3.0, C-1'), 129.6 (d, J = 8.5, C-2'',6''), 134.2, 151.4, 153.9, 157.7, 158.1, 163.7 (d, J = 247.3, C-4'), 164.3. MS, m/z 403.0 [M+H]+.

2-Dichloromethyl-4-(4-fluorophenyl)-7-methylpyrazolo[1,5-a][1,3,5]triazine (3ea). Mp 128–130 °C (MeCN). IR, v, cm−1: 2996, 1604, 1529, 1488, 1233, 1156, 1015, 842, 783, 664, 530. 1H NMR, δ, ppm: 2.51 (3H, s, Me-7), 6.78 (1H, s, H-8), 7.39 (1H, s, CHCl2), 7.47–7.51 (2H, m, H-3',5'), 8.81–8.83 (2H, m, H-2',6'). 13C NMR, δ, ppm (J, Hz): 15.2 (Me-7), 71.4 (CHCl2), 98.1 (C-8), 116.3 (d, J = 21.9, C-3',5'), 126.5 (d, J = 3.0, C-1'), 134.6 (d, J = 10.0, C-2',6'), 150.5, 153.3, 157.8, 159.3, 165.7 (d, J = 253.8, C-4'). MS, m/z 311.0 [M+H]+.

2-Dichloromethyl-4-(4-fluorophenyl)-7-phenylpyrazolo[1,5-a][1,3,5]triazine (3eb). Mp 171–173 °C (MeCN). IR, v, cm−1: 3003, 1603, 1482, 1238, 1156, 1011, 845, 767, 745, 692, 662. 1H NMR, δ, ppm (J, Hz): 7.44 (1H, s, H-8 or CHCl2), 7.48–7.58 (6H, m, H-8 or CHCl2, H-3',5',3''–5''), 8.13 (2H, d, J = 6.8, H-2'',6''), 8.93–8.96 (2H, m, H-2',6'). 13C NMR, δ, ppm (J, Hz): 71.4 (CHCl2), 95.4 (C-8), 116.4 (d, J = 22.4, C-3',5'), 126.5 (d, J = 2.5, C-1'), 127.4, 129.6, 130.8, 131.7, 134.8 (d, J = 9.5, C-2',6'), 151.3, 153.8, 158.1, 159.0, 165.8 (d, J = 253.3, C-4'). MS, m/z 373.2 [M+H]+.

2-Dichloromethyl-4-(4-fluorophenyl)-7-(p-tolyl)pyrazolo[1,5-a][1,3,5]triazine (3ec). Mp 210–212 °C (MeCN+DMF, 2:1). IR, v, cm−1: 3012, 2920, 1603, 1481, 1449, 1223, 1150, 1013, 837, 783, 746, 661. 1H NMR, δ, ppm (J, Hz): 2.39 (3H, s, Me-4'), 7.36–7.61 (6H, m, CHCl2, H-8,3',5',3'',5''), 8.06 (2H, d, J = 8.0, H-2'',6''), 8.95–8.98 (2H, m, H-2',6'). 13C NMR, δ, ppm (J, Hz): 21.5 (Me-4'), 71.4 (CHCl2), 95.1 (C-8), 116.3 (d, J = 22.0, C-3',5'), 126.5 (d, J = 2.9, C-1'), 127.3, 128.9, 130.1, 134.8 (d, J = 9.5, C-2',6'), 140.5, 151.2, 153.5, 158.0, 159.1, 165.7 (d, J = 253.1, C-4'). MS, m/z 387.2 [M+H]+.

2-Dichloromethyl-4,7-di-(4-fluorophenyl)pyrazolo[1,5-a][1,3,5]triazine (3ee). Mp 179–181 °C (MeCN+DMF, 2:1). IR, v, cm−1: 3101, 2999, 2925, 1600, 1480, 1449, 1229, 1152, 845, 789, 742, 660, 553. 1H NMR, δ, ppm: 7.26–7.30 (2H, m, H-3'',5''), 7.39 (2H, br. s, H-8, CHCl2), 7.45–7.48 (2H, m, H-3',5'), 8.08 (2H, br. s, H-2'',6''), 8.86–8.89 (2H, m, H-2',6'). 13C NMR, δ, ppm (J, Hz): 71.3 (CHCl2), 95.2 (C-8), 116.2 (d, J = 22.6, C-3',5''), 116.4 (d, J = 22.1, C-3',5'), 126.2 (d, J = 3.0, C-1'), 128.1 (d, J = 3.0, C-1'), 129.5 (d, J = 8.5, C-2'',6''), 134.7 (d, J = 9.5, C-2',6').
151.2, 153.4, 157.9, 157.9, 163.7 (d, \(J = 233.9, C-4\)”), 165.6 (d, \(J = 239.9, C-4\)’). MS, m/z 391.0 [M+H]+.

4-(4-Chlorophenyl)-2-dichloromethyl-7-methylpyrazolo[1,5-a][1,3,5]triazine (3fa). Mp 162–164 °C (MeCN). IR, \(\nu, \text{cm}^{-1}= 3000, 1599, 1528, 1480, 1235, 1093, 1014, 842, 789, 739, 664, 531. \) 1H NMR, \(\delta, \text{ppm} (J, Hz): 2.52 (3H, s, Me-7), 6.81 (1H, s, H-8), 7.39 (1H, s, CHCl₂), 7.74 (2H, d, \(J = 8.0, H-3′,5′\)), 8.74 (2H, d, \(J = 8.0, H-2′,6′\)). \) 13C NMR, \(\delta, \text{ppm}: 14.7 (\text{Me-7}), 70.9 (\text{CHCl}_2), 97.6 (\text{C-8}), 128.3, 128.7, 132.9, 138.5, 149.9, 152.8, 157.2, 158.8. MS, m/z 329.0 [M+H]+.

4-(4-Chlorophenyl)-2-dichloromethyl-7-phenylpyrazolo[1,5-a][1,3,5]triazine (3fb). Mp 197–199 °C (MeCN+DMF, 4:1). IR, \(\nu, \text{cm}^{-1}= 3020, 2920, 1599, 1478, 1449, 1094, 1014, 840, 783, 744, 662. \) 1H NMR spectrum (CDCl₃), \(\delta, \text{ppm} (J, Hz): 2.42 (3H, s, Me-4’’), 6.73 (1H, s, H-8), 7.01 (1H, s, CHCl₂), 7.29 (2H, d, \(J = 7.8, H-3′,5′\)), 7.29 (2H, d, \(J = 7.8, H-3′,5′\)), 7.90 (2H, d, \(J = 7.8, H-2′,6′\)), 8.99 (2H, d, \(J = 8.4, H-2′,6′\)). \) 13C NMR, \(\delta, \text{ppm}: 21.0 (\text{Me-4’’}), 70.9 (\text{CHCl}_2), 94.6 (\text{C-8}), 126.7, 128.3, 128.5, 130.0, 138.6, 140.0, 150.6, 153.0, 157.4, 158.6. MS, m/z 405.2 [M+H]+.

4-(4-Chlorophenyl)-2-dichloromethyl-7-(4-fluorophenyl)pyrazolo[1,5-a][1,3,5]triazine (3fe). Mp 191–193 °C (MeCN+DMF, 2:1). IR, \(\nu, \text{cm}^{-1}= 3005, 1599, 1478, 1449, 1217, 1158, 1090, 841, 786, 744, 662, 561. \) 1H NMR, \(\delta, \text{ppm} (J, Hz): 7.36–7.40 (2H, m, H-3′,5′”), 7.45 (1H, s, H-8 or CHCl₂), 7.53 (1H, s, H-8 or CHCl₂), 7.79 (2H, d, \(J = 8.8, H-3′,5′\)), 8.18–8.21 (2H, m, H-2′”,6”), 8.84 (2H, d, \(J = 8.8, H-2′”,6”\)). \) 13C NMR, \(\delta, \text{ppm} (J, Hz): 71.3 (\text{CHCl}_2), 95.3 (\text{C-8}), 116.5 (d, \(J = 22.1, C-3′′,5′′\)), 128.2 (d, \(J = 3.0, C-1′′\)), 128.7, 129.2, 129.6 (d, \(J = 8.5, C-2′”,6”\)), 133.5, 139.1, 151.2, 153.8, 158.0, 163.8 (d, \(J = 248.0, C-4′\)). MS, m/z 409.0 [M+H]+.

4-tert-Butyl-2-dichloromethyl-7-(4-methylphenyl)pyrazolo[1,5-a][1,3,5]triazine (3gc). Mp 139–141 °C (MeCN). IR, \(\nu, \text{cm}^{-1}= 3008, 2927, 1599, 1447, 1363, 1245, 839, 791, 748, 659. \) 1H NMR, \(\delta, \text{ppm} (J, Hz): 1.66 (9H, s, Me₃C-4), 2.38 (3H, s, Me-4’’), 7.30–7.36 (4H, m, CHCl₂, H-8,3′′,5′′), 7.99 (2H, d, \(J = 4.0, H-2′”,6”\)). \) 13C NMR, \(\delta, \text{ppm}: 21.0 (\text{Me-4’’}), 26.3 (\text{Me₃C-4}), 38.6 (\text{Me₃C-4}), 70.9 (\text{CHCl}_2), 94.1 (\text{C-8}), 126.7, 128.6, 129.6, 139.9, 149.8, 157.2, 157.4, 165.6. MS, m/z 349.2 [M+H]+.

4-tert-Butyl-2-dichloromethyl-7-(4-fluorophenyl)pyrazolo[1,5-a][1,3,5]triazine (3ge). Mp 93–95 °C (EtOH). IR, \(\nu, \text{cm}^{-1}= 3001, 2934, 1609, 1586, 1511, 1448, 1363, 1254, 1231, 1156, 845, 791, 746, 659. \) 1H NMR, \(\delta, \text{ppm}: 1.66 (9H, s, Me₃C-4), 7.35–7.40 (3H, m, H-8 or CHCl₂, H-3′′,5′′”), 7.45 (1H, s, H-8 or CHCl₂), 8.14–8.18 (2H, m, H-2′”,6””). \) 13C NMR, \(\delta, \text{ppm} (J, Hz): 26.8 (\text{Me₃C-4}), 39.1 (\text{Me₃C-4}), 71.4 (\text{CHCl}_2), 94.8 (\text{C-8}), 116.6 (d, \(J = 21.9, C-3′′,5′′\)), 128.4 (d, \(J = 3.0, C-1′′\)), 129.5 (d, \(J = 8.5, C-2′”,6”\)), 150.4, 156.6, 158.0, 162.8, 165.4 (d, \(J = 247.3, C-4′\)). MS, m/z 353.2 [M+H]+.
In Vitro Anticancer Screening of the synthesized compounds

One Doses Full NCI 60 Cell Panel Assay. The newly synthesized compounds were submitted to National Cancer Institute NCI, Bethesda, Maryland, U.S.A., under the Developmental Therapeutic Program DTP (https://dtp.cancer.gov/discovery_development/nci-60/handling.htm). The cell line panel engaged a total of 60 different human tumor cell lines derived from nine cancer types. The selected compounds 3 were assigned with the NCI codes (see Table 1), respectively Primary in vitro one dose anticancer screening was initiated, in which the full NCI 60 panel lines were inoculated onto a series of standard 96-well microtiter plates on day 0 at 5000–40,000 cells/well in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine, and then preincubated in the absence of drug at 37 °C, and 5 % CO2 for 24 h. Test compounds were then added in the same concentration of 10−5 M in all 60 cell lines (drug solution preparing see in [24]), and incubated for a further 48 h under the same incubation conditions. Following this, the media were removed, the cells were fixed in situ, washed, and dried. The sulforhodamine B assay was used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10 % (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1 % (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

Five Doses Full NCI 60 Cell Panel Assay. All the 60 cell lines, representing nine cancer subpanels (Fig. 1), were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 µM; drug solution preparing see in [23]) of the tested compounds. The outcomes were used to create log10 concentration versus percentage growth inhibition curves and three response parameters (GI50, total growth inhibition (TGI) and LC50) were calculated for each cell line. The GI50 value (growth inhibitory activity) corresponds to the concentration of the compound causing 50 % decrease in net cell growth. The TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition. The LC50 value (cytotoxic activity) is the concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h. Data calculations were made according to the method described by the NCI Development Therapeutics Program.

COMPARE correlations were performed as described in [24]. Vectors of Lg GI50 concentrations for compound 3fa (NSC 811821) were correlated with the set of corresponding average GI50 vectors from the standard agents database or all public NCI-60 vectors that contained at least 40 overlapping cell lines and had SD > 0.2.

Results and Discussion

Chemistry

For synthesis of pyrazolo[1,5-a][1,3,5]triazines 3 we investigated different reaction conditions (at room temperature, under reflux in different solvents, with or without a base catalyst) and the most promising results were achieved when starting reagents were heated
in tetrahydrofuran in the presence of triethylamine. Thus, it has been found that the addition of one equivalent of 1H-pyrazol-5-amines 2 to a stirred solution of N-(2,2-dichloro-1-cyanoethenyl)carboxamides 1 in THF containing one equivalent of triethylamine under reflux gave 2-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazines 3 which were the major products of this one-pot reaction. Apparently, the heterocyclization proceeded in several stages, starting with the addition of an NH2 group to the activated C=C bond to formed intermediate A, followed by the elimination of hydrogen cyanide promoted by triethylamine (and intermediate B creation) with further intramolecular condensation into the final product 3. Recrystallization of crude products easily yielded the pure target compounds. Our method is convenient due to mild reaction conditions, short time of the key reaction, and high degree of purity and good yields of the products.

Compounds 3 are tan solids, melting in the range of 110–210 °C, their structure was established with the help of IR, NMR spectroscopy, mass spectrometry, and X-Ray Analysis of compound 3db (CCDC1920913, deposit@ccdc.cam.ac.uk). 1H NMR signal of CHCl2 and pyrazole CH group occurs in the region 6.7–7.5 ppm. In the spectrum of 3ba, for example, there are two distinguished one proton singlets at 6.80 and 7.40 ppm. For other samples, one or both of these signals overlap with ArH multiplets.

**In Vitro Screening**

**One Doses Assay.** The initial assessment made it possible to identify the eight most promising structures from the collection of synthesized compounds for the one-dose assay: substances 3aa, 3ab, 3ba, 3bb, 3ca, 3fa, 3fb, 3gc. Their results are represented in Table 1.

So, the average value of the effect of the substance 3aa with two methyl substituents on the growth of cancer cells is close to 100 %, and the range of values is also relatively narrow, which indicates its low cytotoxicity. The anticancer properties of substance 3ab with methyl substituent in position 4 of pyrazolo[1,5-a][1,3,5]triazine system and phenyl in 7 are very low too; and some growth inhibition was observed only in the case of line EKVX of non-small cell lung cancer (Table 1). tert-Butyl derivative 3gc also only slightly slows the growth of cancer cells.

However, the presence of aryl substituent (instead of alkyl) in position 4 leads to a swift

| Compound | NCI code | Growth Percent, % |
|----------|----------|-------------------|
| 3aa      | NSC 811824 | 99.5 / 80.6–117.9 |

**Table 1.** The effect of the compounds 3aa, ab, ba, bb, ca, fa, fb, gc on cancer cells growth according to One Doses Full NCI 60 Cell Panel Assay (C = 10−5 mol/L)
| Compound | NCI code | Growth Percent, % mean / range the lowermost values (cell line / panel) |
|----------|----------|---------------------------------------------------------------|
| ![Chemical Structure](image1) | NSC 811820 | 89.6 / 21.5–111.1 21.5 (EKVX / non-small cell lung cancer) 51.6 (H578T / breast cancer) |
| ![Chemical Structure](image2) | NSC 811825 | 49.8 / -50.0 –112.8 -50.0 (NCI-H460 / non-small cell lung cancer) -20.7 (MDA-MB-468 / breast cancer) -14.1 (HL-60(TB) / leukemia) |
| ![Chemical Structure](image3) | NSC 811819 | 5.0 / -82.1–86.9 -82.1 (HCC-2998 / colon cancer) -68.0 (RFX 393 / renal cancer) -58.3 (NCI-H460 / non-small cell lung cancer) -57.0 (ACHN / renal cancer) -52.3 (MDA-MB-468 / breast cancer) |
| ![Chemical Structure](image4) | NSC 811823 | 8.8 / -69.3–81.8 -69.3 (HCC-2998 / non-small cell lung cancer) -66.0 (RFX 393 / renal cancer) -64.8 (NCI-H460 / non-small cell lung cancer) -45.0 (ACHN / renal cancer) |
| ![Chemical Structure](image5) | NSC 811821 | -2.0 / -77.0–67.0 -77.0 (HCC-2998 / non-small cell lung cancer) -74.4 (RFX 393 / renal cancer) -49.4 (NCI-H460 / non-small cell lung cancer) -47.3 (MDA-MB-468 / breast cancer) |
| ![Chemical Structure](image6) | NSC 811822 | 20.9 / -55.0–89.0 -55.0 (RFX 393 / renal cancer) -54.7 (NCI-H460 / non-small cell lung cancer) -53.7 (HCC-2998 / colon cancer) -52.8 (ACHN / renal cancer) -50.5 (NCI-H322M / non-small cell lung cancer) |
| ![Chemical Structure](image7) | NSC 811826 | 84.1 / 35.2–117.5 35.3 (HL-60(TB) / leukemia) 44.5 (NCI-H322M / non-small cell lung cancer) |
increase in activity. Substances 3ba, 3bb, 3ca, 3fa, 3fb can effectively inhibit the growth of certain cancer cell lines. The character of the substituent in position 7 is not so important; and high cytotoxicity is inherent to the substance 3fa with a 7-methyl group and diphenyl derivative 3bb.

Five Doses Assay. According to the results of single dose tests, the most perspective substances 3ba, 3bb, 3ca, 3fa, 3fb were selected for five doses assay to establish the parameters GI\textsubscript{50}, TGI and LC\textsubscript{50}. In Table 2 the mean values of these parameters are given, as well as their values for the cell lines referred in Table 1.

Substances 3ba, 3bb, 3ca, 3fa, 3fb displayed significant growth inhibition effect on cell lines besides those given in Tables 1, 2, as generalized dose response curves demonstrate (Fig. 1).

**Table 2.** The Five Doses Full NCI 60 Cell Panel Assay of the compounds 3ba, bb, ca, fa, fb (the concentrations GI\textsubscript{50}, TGI and LC\textsubscript{50}, mol/L, given as lg)

| Compound / parameter | Mean value NCI-IH460 / non-small cell lung cancer | Value of certain cancer cell lines' growth inhibition |
|----------------------|--------------------------------------------------|----------------------------------------------------|
|                      | Mean NCI-IH460                                  | HCC-2998 / colon cancer                           | RXF 393 / renal cancer |
|                      | lg GI\textsubscript{50}                         | -5.35                                             | -5.71                 | -5.75                 |
|                      | lg TGI                                           | -4.63                                             | -5.29                 | -5.46                 | -5.24 |
|                      | lg LC\textsubscript{50}                         | -4.12                                             | > -4.00              | -5.16                 | -4.65 |
| 3bb (NSC 811819)     | lg GI\textsubscript{50}                         | -5.65                                             | -6.39                 | -6.24                 | -6.27 |
|                      | lg TGI                                           | -4.88                                             | -5.73                 | -5.69                 | -5.62 |
|                      | lg LC\textsubscript{50}                         | -4.18                                             | -5.10                 | -5.28                 | -5.13 |
| 3ca (NSC 811823)     | lg GI\textsubscript{50}                         | -5.68                                             | -6.29                 | -5.93                 | -6.17 |
|                      | lg TGI                                           | -4.68                                             | -5.52                 | -5.59                 | -5.63 |
|                      | lg LC\textsubscript{50}                         | -4.13                                             | -4.12                 | -5.25                 | -5.20 |
| 3fa (NSC 811821)     | lg GI\textsubscript{50}                         | -6.04                                             | -6.52                 | -6.21                 | -6.29 |
|                      | lg TGI                                           | -5.3                                              | -5.97                 | -5.67                 | -5.70 |
|                      | lg LC\textsubscript{50}                         | -4.25                                             | > -4.00              | -5.24                 | -5.27 |
| 3fb (NSC 811822)     | lg GI\textsubscript{50}                         | -5.69                                             | -6.10                 | -6.13                 | -6.09 |
|                      | lg TGI                                           | -4.71                                             | -5.67                 | -5.85                 | -5.73 |
|                      | lg LC\textsubscript{50}                         | -4.36                                             | > -4.30              | -5.56                 | -5.37 |

**NCI 60 Cell Panel COMPARE Correlations**

COMPARE analysis [24] was performed to propose a mechanism of action of the investigated compounds. Only for compound 3fa (NSC 811821) the correlation, computed as the GI\textsubscript{50} vector, exceeded 0.5 in comparison with Fluorouracil. This antineoplastic agent produces active metabolites that incorporate into RNA and DNA and inhibit their processing, thereby inhibiting cell growth [25]. For other investigated compounds (3ba, bb, ca, fb) no analogues of anticancer mechanism were found, therefore 4-(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazine derivatives could potentially be a new class of anticancer agents.

**Conclusions**

By starting from N-(2,2-dichloro-1-cyanoethyl)carboxamides 1 and 1H-pyrazol-5-
Fig. 1. Five Dose Data Graphs for compounds 3ba, bb, ca, fa, fb log₁₀C (C – compound concentration, mol/L) / cancer cells percentage growth, %
amines 2, 26 new 4(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazine derivatives with expected biological activity were synthesized. Compounds 3ba, 3bb, 3ca, 3fa, 3fb with an aromatic substituent in position 4 can effectively inhibit the growth of certain cancer cell lines, whereas compounds with an alkyl substituent in the same position possess low cytotoxicity. Future work will be focused on the improvement of their biophysical properties to yield drug-like pre-clinical candidates for in vivo animal studies. COMPARE analysis did not reveal any known anticancer drugs with a similar action, which warrants a more detailed study of the anticancer action mechanism of the obtained 4(dichloromethyl)pyrazolo[1,5-a][1,3,5]triazine.

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вої активності показало, що 5 з 26 досліджуваних сполук можуть ефективно пригнічувати ріст певних ракових клітинних ліній. Висновки. Новий тип гетероциклизacji N-(2,2-дихлоро-1-ціаноэтил)карбоксамідів з 1H-піразол-5-амінами привела до отримання 2-(дихлорометил)піразоло[1,5-a][1,3,5]триазинів. Деякі з отриманих сполук пригнічують ріст певних ракових клітин.

Ключові слова: in vitro скринінг, протиракова активність, гетероциклизация, 1H-піразол-5-аміни, піразоло[1,5-a][1,3,5]триазини, 2-(дихлорометил)піразоло[1,5-a][1,3,5]триазини.

2-(Дихлорометил)піразоло[1,5-a][1,3,5] триазини: синтез і противоракова активність

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Цель. Синтез серії 2-(дихлорометил)піразоло[1,5-a][1,3,5]триазинів і вивчення їх противоракової активності in vitro на панелі з 60 клеточних ліній,

получених із 9 типів рака, а іменно лейкемії, немелкоклеточный рак легких, рак толстой кишки, рак ЦНС, меланома, рак яичников, рак почек, рак простата, рак молочної жєлези. Методы. Органический синтез; біологічні тести; спектральний аналіз; статистичні методи. Результаты. Скрининг протиракова активності in vitro показав, що 5 з 26 ісследованих сполук можуть ефективно інгібувати ріст певних ліній ракових клеток.

Выводы. Новий тип гетероциклизации N-(2,2-дихлоро-1-ціаноэтил)карбоксамідів і 1H-піразол-5-амінів привел до отримання 2-(дихлорометил)піразоло[1,5-a][1,3,5]триазинів. Некоторые із отриманих соединений ингибируют рост определенных линий раковых клеток.

Ключевые слова: in vitro скрининг, противораковая активность, гетероциклизация, 1H-піразол-5-аміни, піразоло[1,5-a][1,3,5]триазини, 2-(дихлорометил)піразоло[1,5-a][1,3,5]триазини.

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