A Novel Synthesis Of Fused Pyrazolopyrimidine: Pyrazolo-Triazolo-Pyrimidine For Anticancer Evaluation.

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Abstract: Some novel fused pyrazolo-triazolo-pyrimidine (2-5) were designed and prepared via reaction of 5-amino-6-hydrazinyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (1) with some electrophilic and nucleophilic reagents. The structures of target compounds were confirmed by elemental analyses and spectral data. The newly synthesized compounds were selected by the NCI – Maryland-U.S.A. and were tested for their anticancer activity in an initial single high dose in the full NCI 60 cell line panel.

Keywords: pyrazolo [3,4-d] pyrimidine, pyrazolotriazolopyrimidine, anticancer activity.

1. Introduction

Pyrazolo[3,4-d]pyrimidines and their correlating fused heterocycles are of significant chemical and pharmaceutical interest as purine parallel [1] and abundant of their derivatives were reported to have antiviral, [2,3] anticancer, [4] anti-inflammatory, [5,6] antimycobacterial, [7] antimicrobial, [8,9] activities and xanthine oxidase inhibitor [7] like allopurinol which was first synthesized by Robins in 1956,[10] and is still the drug for the treatment of hyperuricemia and gouty arthritic disease.[11] Moreover, pyrazolotriazolopyrimidines expected to possess notable chemical and pharmacological activities [8,12–15] and represented an attractive key intermediate for obtaining and selective human A3 adenosine receptors. [14] Therefore, based on the previous reports we interested in this paper on the synthesis of fused pyrazolo-triazolopyrimidine derivatives and exploring novel anticancer agents of some new compounds conserving the pyrazolopyrimidine nucleus.

2. Results and discussion

Synthesis of the objective compounds was carried out according to the proceedings light in schemes 1. As explained in Figure 1 treating of 5-aminopyrazole-4-carboxylate with thiocarboxyhydrate (TCH) in ethanolic sodium ethoxide solution under reflux confer compound 1 as the start of all preparations. $^1$HNMR spectrum of 1 showed five deuterium exchangeable signals at $\delta$ (5.29, 5.69, 6.29, 6.32, 8.19) ppm due to $\equiv$NH$_2$(tautomer), NH-pyrimidine(tautomer), NH$_2$, NH$_2$(tautomer) and NHNH$_2$ protons, respectively. Cyclocondensation of 1 with ethyl pyridine-2-carboxylate or 2,2,2-trichloro-acetamide under refluxing in ethanolic sodium ethoxide solution produced compound 2 with releasing molecules of H$_2$O and EtOH, while resulted compound 3 with elimination of H$_2$O and NH$_3$ molecules, respectively. IR and $^1$HNMR of 2 and 3 showed no signal for NH of 6-hydrazinyl pyrimidine and this an evidence on cyclization. $^{13}$C NMR spectrum of 3 showed signals at $\delta$ (87.0, 156.0) ppm assignable to CCl and C=N triazole carbons, respectively besides the expected signals for aromatic carbons. The mass spectra of 2 and 3 also support their chemical formula and showed a molecular ion peak at $m/z = 344$ (M$^+$) and $m/z = 383$(M$^+$ - 1), respectively. Fusion of 1 with salicylaldehyde produced compound 4 after removal H$_2$O molecule. IR spectrum of 4 showed peaks at (3424, 3320) cm$^{-1}$ for OH and NH$_2$ groups, its $^1$HNMR spectrum revealed two deuterium exchangeable signals due to NH-triazole and OH moieties at $\delta$ (8.70, 9.90) ppm, respectively, also displayed a singlet signals at $\delta$ 4.17 ppm for OH and NH$_2$ group.

Also, its $^{13}$CNMR spectrum showed signals at $\delta$ 85.3 ppm assignable to C$_{12}$ triazole carbon besides the expected signals for aromatic and others carbons. The mass spectrum of 4 also support its chemical formula and showed a molecular ion peak at $m/z = 361$ (M$^+$). The reaction of 1 with cyanamide by fusion can be occurred according to nucleophilic addition of cyamanide NH$_2$ on carbonyl oxygen of 1 and elimination of H$_2$O molecule followed by nucleophilic attack of NH$_2$ pyrimidine on cyanamide C=N which lead to cyclization and formation of compound 5. The IR spectrum of 5 showed the absence of the amidic carbonyl group. Also, its $^{13}$CNMR spectrum confirmed the structure as there are no signal for C=O and showed a signal assignable to C=N triazole carbon at $\delta$ 161.1 ppm besides the expected signals for aromatic carbons. The mass spectrum of 5 also support its chemical formula and showed a molecular ion peak at $m/z = 281$ (M$^+$). (Scheme 1)
3. Biological evaluation

3.1. NCI results.

The synthesized Compounds were selected by the National Cancer Institute (NCI, Bethesda, USA) at one dose (10^3 M) in the full 60 human cancer cell lines orderly into subpanels derived from nine various human cancer forms: leukemia, melanoma, lung, colon, renal, ovarian, breast, prostate and CNS. The anticancer activity of the synthesized compounds is presented in Table (1).

4. Conclusion

Compounds 2, 3, 4 and 5 were synthesized by the reaction of 5-amino-6-hydrazinyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one 1 with some electrophilic and nucleophilic reagents by refluxing or fusion and they exerted mild anticancer activity against certain cancer cell lines.

5. Experimental

5.1. Chemistry

Melting points were determined on Electro thermal LA 9000 SERIS. Infrared (IR) spectra were recorded on Schimadzu FT-IR Affinity-1 spectrometer. 1H, 13C NMR spectra were recorded on JEOI spectrometer (500 MHz) and Bruker high performance digital FT-NMR spectrometer avance III (400 MHz) using deuterated dimethylsulfoxide (DMSO-d6) as a solvent. The Mass spectra were carried out using a Schimadzu GC/ MS-QP-5050A mass spectrometer at 70 eV in the regional center for myology and biotechnology, at Al-Azhar University.

5.1.1.5-amino-6-hydrazinyl-1-phenyl-1,5-dihydro-4H-pyrazolo [3,4-d] pyrimidin-4-one&/D(5) 1/5-amino-6-hydrazinylidene-1-phenyl-1,5,6,7-tetrahydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (1, C12H15N3O) An equimolar mixture of 5-amino pyrazole-4-carboxylate (2.31g, 10 mmol) and thio carbonyldiimide (TCH) (1.06g,10 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium ethoxide (0.24 g, 10 mmol) in absolute ethanol (30 ml)) was heated under reflux for 8 h. The reaction mixture was cooled, poured on to crushed ice, the precipitate was collected by filtration to afford compounds 2 and 3, respectively.

5.1.2. 8-amino-1-phenyl-7-(pyridin-2-yl)-1,8-dihydro-4H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a] pyrimidine-4-one(2, C17H15N3O), white solid, crystallized from acetone (2.03 g, 59%). M.p.: 234-236°C. IR (KBr, cm⁻¹): 3432, 3322(NH2); 2049; CH-aromatic); 1618(C=N); 1548(C=C). 1H NMR (500 MHz, DMSO-d6): 6.28 (2H, NH2, D2O exchangeable);7.38-7.66 (m, 9H, Ar-H); 8.18 (s, 1H, =CH-pyrazole). MS (m/z, %): 344.04 (M⁺, 17); 56.48 (100). Anal. Calcd for C17H15N3O (344.34); C, 59.30; H, 3.51; N, 32.54. Found: C, 59.41; H, 3.58; N, 32.63.

5.1.2.2. 8-amino-1-phenyl-7-(trichloromethyl)-1,8-dihydro-4H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a] pyrimidine-4-one (3, C13H11Cl2N3O), white solid, crystallized from ethanol (1.99 g, 52%). M.p.: 144-146°C. IR (KBr, cm⁻¹): 2341, 3222(NH2); 3048(CH-aromatic); 1660(C=O-amidic); 1618(2C=N); 1547(C=C). 1H NMR (500 MHz, DMSO-d6): 6.20 (s, 2H, NH2, D2O exchangeable) 7.38-7.55 (m, 5H, Ar-H); 8.13 (s, 1H, =CH-pyrazole). 13C NMR (500MHz, DMSO-d6): 87.0(C15-C11); 112.0(C4-C1 fused pyrazolopyrimidine); 124.1, 126.0, 129.6, 139.2 (phenyl C). IR (KBr, cm⁻¹): 3835.52 (M⁺ - 1, 17); 185.08 (100). Anal. Calcd for C13H11Cl2N3O (384.61); C, 40.60; H, 2.10; Cl, 27.65; N, 25.49. Found: C, 40.63; H, 2.17; Cl, 27.74; N, 25.52.

5.1.3. General method for the preparation of compounds 4 and 5.

An equimolar mixture of compound 1 (2.57g, 10 mmol) and salicylaldehyde (1.22g, 1.06ml) or cyanamide (0.42g, 0.3049(CH=O)) was heated at 200°C for 6 h. The fused mass thus obtained was treated with ethanol, collected by filtration and washed with ethanol, methanol, ethyl acetate, acetone and hexane to afford compound 4 and 5, respectively.

5.1.3.1.8-amino-7-(2-hydroxyphenyl)-1-phenyl-1,6,7,8-tetrahydro-4H-pyrazolo [3,4-d][1,2,4] triazolo[1,5-a] pyrimidine-4-one (4, C18H15N3O), brown powder (2.02g, 56%). M.p.: >360 °C. IR (KBr, cm⁻¹): 3432,3322(OH2, NH2); 3220(NH) 3068(CH-aromatic); 2962 (CH-aliphatic); 1650 (C=O amide); 1624(2C=N); 1597(C=C). 1H NMR (400 MHz, DMSO-d6): 4.17 (s, 1H, CH-triazole); 4.42 (s, 2H, NH2D2O exchangeable) 7.02-7.63 (m, 9H,Ar-H); 8.35 (s, 1H, =CH-pyrazole); 8.70 (s, 1H, NH,D2O exchangeable) 9.90 (s, 1H, OH, D2O exchangeable). 13C NMR (500MHz, DMSO-d6): 85.3(C12-C11);110.0(C14-C13);115.5, 122.7, 129.0, 129.3, 141.0, 154.2 (phenyl C); 147.5(C10); 141.0 (C5-spyrazole); 152.3(C2=C=N pyrimidine); 160.8(C=O). MS (m/z, %): 361.23 (M⁺, 9); 158.77 (100). Anal. Calcd for C18H15N3O (361.37); C, 59.83; H, 4.18; N, 27.13. Found: C, 59.89; H, 4.22; N, 27.18.

5.1.3.2.5-hydrazinyl-7-phenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-cyrimidin-2-amine (5, C5H12N5O), white solid (1.51g, 54%). M.p.: >360°C. IR (KBr, cm⁻¹): 3334,3149,3120 (NH2, NH); 3060 (CH-aromatic);1631 (4C=N); 1540 (C=C). 1H NMR (400 MHz, DMSO-d6): 6.15 (s, 2H, NH2, D2O exchangeable); 6.97 (s, 3H, NH2H2, D2O exchangeable); 7.12-7.91 (m, 5H,Ar-H); 8.04 (s, 1H, =CH-
pyrazole); 10.40 (s, 1H, NH (automer), D₂O exchangeable). \(^{13}\)C NMR (500MHz, DMSO-d₆): 104.2 (C₆-fused pyrazolopyrimidine); 118.5, 126.1, 128.2, 139.4 (phenyl C₁₄–₁₉); 136.0 (C₇=N, pyrazole); 148.1 (C₁₀-fused pyrazolopyrimidine); 161.1 (C₃=N, triazole); 169.3 (C₁₂=N, pyrimidine). MS (m/z, %): 281.04 (M⁺, 7); 43.14 (100).

Anal. Calcd for C₁₂H₁₁N₉ (281.28); C, 51.24; H, 3.94; N, 44.82. Found: C, 51.31; H, 3.97; N, 44.85.

**Figure 1.**

**Scheme (1)**

5.2. NCI methodology

All compounds submitted to the NCI 60 Cell screen are tested at a single high dose (10⁻⁵ M) as previously reported[16].

**Acknowledgments**

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Table (1): Evaluation of anticancer activity (Percentage of Growth inhibition) of the selected compounds from scheme 1 for most sensitive cell lines.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>NCI-No.</th>
<th>Mean Growth %</th>
<th>Range of Growth %</th>
<th>Subpanel cell line (Growth inhibition percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>812489</td>
<td>100.14</td>
<td>40.38 – 79.56 to 119.94</td>
<td>CNS Cancer: SNB-75 (20.44). Renal Cancer: CAKI-1 (16.88), UO-31 (17.30).</td>
</tr>
<tr>
<td>3</td>
<td>812490</td>
<td>100.05</td>
<td>55.26 – 81.01 to 136.27</td>
<td>CNS Cancer: SNB-75 (15.00). Ovarian Cancer: SK-OV-3 (15.39). Renal Cancer: CAKI-1 (16.09), UO-31 (18.99).</td>
</tr>
<tr>
<td>5</td>
<td>812438</td>
<td>100.37</td>
<td>54.05 – 68.58 to 123.13</td>
<td>Ovarian Cancer: IGROV1 (31.42). Renal Cancer: CAKI-1 (16.08), UO-31 (30.79).</td>
</tr>
</tbody>
</table>

Range: Variation in value between the most sensitive cell lines and the most inefficient one.

References


