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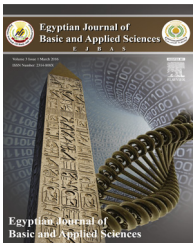

Full Length Article

Synthesis and antitumor activity of some new pyrazolo[3,4-d]pyrimidine and pyrazolo[3,4-b]pyridine derivatives

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ABSTRACT

A new series of pyrazolo[3,4-d]pyrimidine-3-carbonitrile and pyrazolo[3,4-b]pyridine-3-carbonitrile derivatives was synthesized by the reaction of 5-amino-1-tosyl-1*H*-pyrazole-3,4-dicarbonitrile as a key starting material with various electrophilic and nucleophilic reagents. All the newly synthesized compounds were structurally confirmed by various modern analytical methods (IR, ^{1}H NMR and MS). All the title compounds have been evaluated for their potential cytotoxicity against human laryngeal epidermoid carcinoma cells **(Hep2)**. Among the screened compounds, **3** and **4** exhibited the highest significant effect.

1. Introduction

Pyrazole derivatives are characterized by their biological and pharmacological activities as potential inhibitors of HIV-1 [1], pesticides [2], fungicides [3], antihypertensive agents [4] and anticancer activity [5]. They are also important and useful precursors for the synthesis of other fused heterocyclic systems, among these pyrazolo[3,4-d]pyrimidine derivatives [6], which have a considerable chemical and pharmacological importance as purine analogues [7–9]. In the literature, it was found that the replacement of 1*H* of pyrazole of pyrazolo[3,4-d]pyrimidine ring system by some other bioactive moieties drastically alters its pharmacological properties [10]. Also, the pyrazolo[3,4-*b*]pyridine derivatives represent important building blocks in both natural and synthetic bioactive compounds [11]. They show anxiolytic activity along with xanthine oxidase inhibitors, cholesterol formation inhibitor, and anti-Alzheimer [12]. Moreover, fused heterocyclic containing pyrazolopyridine systems have been described to be associated with several biological and medicinal activities [13–16]. In the present work, some new 1-tosyl-pyrazolo[3,4-d]pyrimidine and 1-tosyl-pyrazolo[3,4-b]pyridine

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derivatives incorporating the tosyl moiety were synthesized to evaluate their potential cytotoxicity against epidermoid carcinoma of the larynx (**Hep2**).

2. Experimental

2.1. *Materials and methods*

All melting points (uncorrected) were determined on an electrothermal Gallenkamp melting point apparatus. The IR spectra were recorded in KBr disks on a Thermo Scientific Nicolet iS 50 FT-IR spectrometer (not all frequencies are reported). The NMR spectra were acquired using a Bruker WP 300 spectrometer at 300 MHz using *TMS* as an internal standard and DMSO-d_6 as solvent. The mass spectra were performed using a LC-MS (Shimadzu-Mass spectrometer) at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Cairo University, Giza, Egypt; the results were in satisfactory agreement with the calculated values.

2.1.1. *5-amino-1-tosyl-1H-pyrazole-3,4-dicarbonitrile (2)*

To a cold solution of p-toluenesulfonyl hydrazide (0.05 mol, 9.3 g) in 50 ml ethanol, tetracyanoethylene (0.05 mol, 6.4 g) was added. The reaction mixture was stirred for 1 hour and then heated under reflux on a steam bath for 30 minutes. The mixture was then cooled and the white precipitate was collected and washed with ethanol.

Yield 80%; mp (°C); 213–215 (Lit. m.p. = 214–216 °C) [17]; IR ($\bar{V}/cm^{-1}$): 3313, 3246 (NH_2), 2238 (C—N), 1639 (C—N); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.30 (s, 3H, CH_3), 7.10 (d, 2H, Ar-H, J = 7.95 Hz), 7.50 (d, 2H, Ar-H, J = 1.80 Hz), 7.85 (s, 2H, NH_2); MS (EI): m/z (%) = 287 (molecular ion, 19), 272 (14), 242 (7), 215 (33), 195 (8), 173 (15), 155 59), 139 (6), 114 (37), 91 (base peak, 100), 77 (23), 65 (40). Analysis for $C_{12}H_9N_5O_2S$ (287.30): Calcd.%: C, 50.17; H, 3.16; N, 24.38; Found%: C, 50.28; H, 3.11; N, 24.45.

2.1.2. *4,5,6,7-tetrahydro-4,6-dithioxo-1-tosyl-1H-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (3)*

To a stirred suspension of compound **2** (2 mmol, 0.57 g) in pyridine (20 ml), carbon disulfide (4 mmol, 0.3 ml) was added dropwise. The reaction mixture was then heated on water bath for 12 hrs. The reaction mixture was cooled at room temperature, then poured into ice cold water, and neutralized with hydrochloric acid. The precipitated product was filtered off, washed and recrystallized from EtOH-DMF mixture (1:1) to afford the corresponding product **3** as dark green crystals.

Yield 61%, mp (°C); > 300; IR ($\bar{V}/cm^{-1}$): 3437 (NH), 2221 (C—N), 1638 (C—N); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.25 (s, 3H, CH_3), 7.10 (d, 2H, Ar-H, J = 7.95 Hz), 7.50 (d, 2H, Ar-H, J = 1.80 Hz), 11.22 (s, 1H, NH), 13.69 (s, 1H, NH); MS (EI): m/z (%) = 363 (molecular ion, 20.1), 200 (21.2), 172 (17.7), 133 (48.1), 91 (188.7), 80 (base peak, 100), 64 (63.7). Analysis for $C_{13}H_9N_5O_2S_3$ (363.44): Calcd.%: C, 42.96; H, 2.50; N, 19.27; Found%: C, 42.82; H, 2.56; N, 19.35.

2.1.3. *4-Imino-5-phenyl-6-thioxo-1-tosyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (4)*

A mixture of compound **2** (2 mmol, 0.57 g) and phenyl isothiocyanate (2 mmol, 0.24 ml) in 15 ml DMF was refluxed for 12 hrs. The reaction mixture was allowed to cool at room temperature and then poured into ice cold water. The precipitate that formed was filtered off, dried and then recrystallized from EtOH to afford **4** as yellowish brown crystals.

Yield 65%; mp (°C); 155–157; IR ($\bar{V}/cm^{-1}$): 3374 (NH), 3180 (NH), 2222 (C—N), 1632 (C—N); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.35 (s, 3H, CH_3), 6.90–7.35 (m, 7H, Ar-H), 7.65 (d, 2H, Ar-H, J = 1.85 Hz), 10.25 (s, 1H, NH), 12.45 (s, 1H, NH); MS (EI): m/z (%) = 422 (molecular ion, 6), 372 (17), 345 (20), 329 (31), 311 (41), 297 (44), 282 (35), 268 (46), 253 (base peak, 100), 236 (65), 210 (19), 182 (18), 155 (11), 133 (26), 107 (33), 93 (60), 77 (65), 65 (20). Analysis for $C_{19}H_{14}N_6O_2S_2$ (422.48): Calcd.%: C, 54.01; H, 3.34; N, 19.89; Found%: C, 54.18; H, 3.41; N, 19.78.

2.1.4. *4-Amino-6-thioxo-1-tosyl-6,7-dihydro-1H-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (5)*

A mixture of compound **2** (2 mmol, 0.57 g) and thiourea (2 mmol, 0.16 g) was heated together in oil bath at 180–185 °C for 2 hrs. After cooling, the resulting solid was dissolved in dilute sodium hydroxide and then acidified with dil. HCl to give the crude product **5**. The solid product that formed was filtered off, dried and then recrystallized from EtOH to furnish **5** as dark yellow crystals.

Yield 65%; mp (°C); > 300; IR ($\bar{V}/cm^{-1}$): 3337, 3193 (NH and NH_2), 2221 (C—N), 1640 (C—N); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.35 (s, 3H, CH_3), 6.40 (s, 2H, NH_2), 7.25 (d, 2H, Ar-H, J = 8.05 Hz), 7.65 (d, 2H, Ar-H, J = 1.80 Hz), 11.25 (s, 1H, NH); MS (EI): m/z (%) = 346 (molecular ion, 9.1), 246 (40.2), 172 (21.0), 133 (19.9), 107 (32.6), 91 (base peak, 100), 77 (32.0), 65 (41.7). Analysis for $C_{13}H_{10}N_6O_2S_2$ (346.39): Calcd.%: C, 45.08; H, 2.91; N, 24.26; Found%: C, 45.29; H, 2.80; N, 24.38.

2.1.5. *4,5-Diamino-6-thioxo-1-tosyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (6)*

A mixture of compound **2** (2 mmol, 0.57 g) and thiosemicarbazide (2 mmol, 0.19 g) was fused in oil bath at 180–185 °C for 3 hrs under dry conditions. The fused mixture was heated in ethanol (15 ml) and the precipitate that formed on cooling was collected by filtration, dried and recrystallized from EtOH:DMF mixture (1:1) to furnish compound **6** as brown crystals.

Yield 55%; mp (°C); > 300; IR ($\bar{V}/cm^{-1}$): 3417, 3332, 3195 (NH and NH_2), 2218 (C—N), 1632 (C—N); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.35 (s, 3H, CH_3), 5.20 (s, 1H, CH), 6.15 (s, 2H, NH_2), 7.25 (d, 2H, Ar-H, J = 8.10 Hz), 7.70 (d, 2H, Ar-H, J = 1.80 Hz), 8.35 (s, 2H, NH_2), 11.10 (s, 1H, NH); MS (EI): m/z (%) = 363 (molecular ion, 20.0), 304 (29.2), 266 (45.4), 246 (68.4), 190 (64.8), 172 (28.7), 133 (17.0), 107 (34.6), 91 (100.0), 77 (35.6), 65 (44.8). Analysis for $C_{13}H_{13}N_7O_2S_2$ (363.42): Calcd.%: C, 42.97; H, 3.61; N, 26.98; Found%: C, 43.14; H, 3.72; N, 26.86.

2.1.6. *4-Oxo-1-tosyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (7)*

A suspension of compound **2** (2 mmol, 0.57 g) in excess of formic acid (10 ml) was refluxed on sand bath for 6 hrs. After cooling, the mixture was diluted by cooled water (10 ml) and the precipitate that formed was filtered off, dried and purified by recrystallization from EtOH to give **7** as yellowish brown powder.

Yield 55%; mp (°C); 266–268; IR ($\bar{V}/cm^{-1}$): 3253 (NH), 2240 (C—N), 1668 (C—O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.40 (s, 3H, CH_3),