Synthesis and anticancer evaluation of some novel pyrimido[5,4-e][1,2,4]triazines and pyrazolo[3,4-d]pyrimidine using DMF-DMA as methylating and cyclizing agent

Samar A. El-Kalyoubi1,2*

Abstract

Background: Described a series of main target compounds pyrimido[5,4-e][1,2,4]triazines is obtained via condensation of 6-hydrazinyluracil with different aromatic aldehydes to give the hydrazones followed by nitrosation with HNO2 then intramolecular cyclization. On the other hand, pyrazolopyrimidines can be obtained by the reaction of hydrazones with dimethylformamide-dimethylacetal (DMF-DMA), DMF-DMA in the presence of DMF or by refluxing the hydrazinyluracil with DMF-DMA in the presence of DMF directly. The newly synthesized compounds are evaluated in vitro for their anticancer activity against human lung carcinoma (A549).

Results: A newly substituted compounds of benzaldehyde-pyrimidin-4-yl)hydrazones (5a–f), pyrimido[5,4-e][1,2,4]triazines 6a–e, arylethinylidenehydrazinylpyrimidine 7ab and pyrazolopyrimidines 9,11 are screened for cytotoxic activity against human lung carcinoma (A549) cell line. They exhibited a good yield. Compound 6b shows the highest effect with IC50 value 3.6 μM, followed by compounds 9, 5a, 8, 5e, 6e, 5b, 5f, 7a, 5c, 6c, 7b, 6a, 11, 5d and 6d.

Conclusion: A simple and efficient route is used for the synthesis of pyrimido[5,4-e][1,2,4]triazines and pyrazolopyrimidines. The synthesized compounds are screened for antitumor activity.

Keywords: 6-Hydrazinyluracil, Pyrimidotriazine, Pyrazolopyrimidine, Dimethylformamide-dimethylacetal, Anticancer activities

Background

Triazine is analogues of six membered benzene ring via replacing the three carbon atoms with nitrogens. 1,2,4-triazine and their fused ring structures with one or more heterocycles represent an important class of nitrogen heterocycles compounds. It possess the motif part of naturally and synthetic pharmaceutical products [1–9]. They exhibit a broad spectrum biological effects [10] with antibacterial [11, 12], antitumor [13, 14], anti-convulsant [15], anti-inflammatory [16], and antiviral properties [17]. 6-Azacytosine and 6-azauracil are used as effective antiviral and antitumor activities [18–21]. The tirapazamine (TPZ) is efficacious in the treatment of different human cancer cells via inducing DNA damage in poorly oxygenated tumor cells [22].
The pyrimidotriazine antibiotics represent a wide spectrum of both antimicrobial and antitumor activities [23]. Pyrimido[5,4-e][1,2,4]triazine constitutes the essential active ingredient of the antibiotics like fervenulin (which is formed from actinomycetes), xanthothricin, and reumycin [2, 3]. Reumycin [24] is isolated from actinomycetes rectus bruneus and used as an antitumor antibiotic for treating brain tumors. Other hetero annelated 1,2,4-triazines have clinical antiviral effect against influenza A and B viruses [4], anti-HIV and anticaner activity [5, 6]. They also show antimicrobial, antifungal effects and cytotoxicity to MCF-7 cells [7, 8]. Fervenulin (planomycin), and its tautomeric isomer toxoflavin (panthothricin) reveal a wide spectrum antibacterial, antifungal, herbicidal and anticaner activities [25–27].

**Result and discussion**

**Chemistry**

In continuation to our research, the importance of fervenulin and its diverse pharmacological activity on the medical field, especially as antitumors, we became interested in the prospect of developing our strategies to synthesize new fervenulin analogues of pyrimidotriazine and pyrazolopyrimidine derivatives using 6-hydrazinyl-1-propyluracil (4) as a core for construction. This substrate is prepared via simple hydrolysis of 2,4,6-trichloropyrimidine [37] followed by N-1 selective alkylation using propyl iodide in DMSO in the presence of potassium carbonate as a basic medium [38] then hydrazinolysis of 6-chloro-1-propyluracil (3) with hydrazine hydrate [38–40]. Condensation of substrate 4 with different aromatic aldehydes in ethanol at room temperature for 1 h leads to the formation of hydrazones 5a–f in a good yield (Scheme 1).

The IR spectra of hydrazones displayed the N–H stretching bands at 3271–3122 cm$^{-1}$. The stretching band of the two C=O groups (Amide I) is displayed within the range 1740–1625 cm$^{-1}$. Compound 5d showed O–H stretching bands at 3560 cm$^{-1}$ while the nitro group in compound 5e shows strong asymmetric and symmetric NO$_2$ stretching bands at 1514 and 1337 cm$^{-1}$, respectively. The $^1$H-NMR spectra supported the previous observation from the IR spectra, where N3–H and C6–NH is highly deshielded. They appeared around $\delta$ 10.75–10.05 ppm, while the $\alpha$-CH of hydrazone appeared at the range $\delta$ 8.48–8.24 ppm. The C5–H was the most
shielded as expected around δ 5.46–5.30 ppm. The downfield shift of the α-carbon of hydrazone appeared around δ 145 ppm in the 13C-NMR spectra.

Pyrimidotriazines 6a–e is isolated by the nitrosation of hydrazone compounds 5a–e at C-5 with in situ prepared nitrous acid. The inseparable 5-nitroso-derivatives undergoes cyclization via the nucleophilic attack of the electron rich α-carbon of the hydrazones on the nitroso group to form hydroxylamine intermediates, which are converted into the target pyrimidotriazines 6a–e by protonation of the N-hydroxyl group followed by the elimination of H3O+ (Scheme 2). The IR spectra of 6a–e displayed broad absorption bands of NH stretching in the region of 3180–3135 cm⁻¹. The two bands of C=O groups gave rise in the region of 1725–1670 cm⁻¹.

Moreover, the cyclization of the hydrazone series are confirmed in 1H-NMR spectra through the disappearance of both the α-CH hydrazone at δ 8.48–8.24 ppm and the C5-H of uracil at δ 5.46–5.30 ppm.

Scheme 1 Reaction of 6-hydrazinyluracil with different aromatic aldehydes and formation of pyrimidotriazines. a = NaOH/H2O/Reflux; b = Pri/K2CO3/DMSO; c = NH2NH2.H2O/rt; d = ArCHO/EtOH/rt; e = NaNO2/AcOH/Reflux
Another condensation reaction is obtained via condensation of 4 with different acetophenones by stirring at room temperature for 3–4 h (Scheme 3). The IR spectra of 7a,b displayed stretching bands at the range of 3188–3154 cm$^{-1}$ due to N–H absorption and characteristic bands at the range 1715–1691 cm$^{-1}$ due to absorption of C$=$O groups. The mass spectra of these compounds show the expected molecular ions, whereas their $^1$H-NMR spectra exhibited two signals at $\delta$ 11.11–11.06 ppm and at $\delta$ 9.13–8.96 ppm ascribed for N3–H and C6–NH protons respectively. The singlet signals of the methyl group protons at the $\alpha$-carbon appeared at $\delta$ 2.42–2.37 ppm while for the CH-5 position appeared at $\delta$ 5.48–5.39 ppm. $^{13}$C-NMR confirmed the structure of 7a,b where the key signals at $\delta$ 78.8–79.8 ppm and $\delta$ 14.3–14.2 ppm are assigned to $sp^2$ carbon at position 5 and $sp^3$ carbon attached to the $\alpha$-carbon respectively.

The target compound 9 is prepared by refluxing of 7a with DMF-DMA for 12 h or DMF-DMA in presence of DMF as a solvent for 1 h (Scheme 3). DMF-DMA is a convenient electrophile to introduce one-carbon units. The reaction proceeds via nucleophilic attack of C-5 to electrophilic carbon center of acetal in DMF-DMA followed by intramolecular cyclization and elimination of dimethylamine. A subsequent methylation of NH-5 is observed which arises from O–CH$_3$ group of the acetal not N–CH$_3$ as illustrated in Scheme 4. The plausible mechanism is proved by isolation of the intermediate 8. This intermediate is easily identified in IR, Mass, $^1$H-NMR and $^{13}$C-NMR spectra. A broad stretching absorption band of NH appears at the region of 3136 cm$^{-1}$ of the intermediate 8 in IR spectra and disappears in the target compound 9. Furthermore, $^1$H-NMR showed the disappearance of CH-5 proton at $\delta$ 5.48–5.39 ppm and the appearance of a
singlet signal at $\delta$ 8.08 ppm characteristic for CH–N proton, a singlet signal of $\alpha$-C–CH$_3$ at $\delta$ 2.39 ppm and two characteristic N–CH$_3$ group at $\delta$ 3.19, 3.05 ppm of compound 8 and disappears in compound 9 due to the elimination of dimethylamine (Scheme 4).

Whereas, the alkylation on N-5 in compound 9 is proven without doubt by the disappearance of a singlet signal of NH-5 proton at $\delta$ 10.13 ppm and the appearance of the $sp^3$ singlet signal at $\delta$ 3.23 ppm characteristic of CH$_3$ appears in $^1$H-NMR and a signal at $\delta$ 27.6 ppm in $^{13}$C-NMR. In addition, the $^1$H-NMR shows a singlet signal of CH-3 at $\delta$ 8.60 ppm and two doublet signals at $\delta$ 5.71–5.61 ppm corresponding to the two protons of methylene group which indicates that they are not magnetically equivalent. $^{13}$C-NMR shows the appearance of signals at $\delta$ 134.2 and 101.7 ppm characteristic for C-3 and methylene carbon atom respectively.

Treatment of 4 with DMF-DMA in the presence of DMF by refluxing for 1 h yielded compound 11 (Scheme 3). IR spectra shows characteristic absorption band at 1751, 1698 cm$^{-1}$ for C=O groups. $^1$H-NMR spectrum displays three singlet signals at $\delta$ 8.41, 3.88 and 3.19 ppm for CH-3, N(2)–CH$_3$ and N(5)–CH$_3$ respectively. On the other hand, $^{13}$C-NMR showed C–N(2) at $\delta$ 40.4 ppm and C–N(5) at $\delta$ 27.5 ppm which confirms the alkylation of N(5) with DMF-DMA. The plausible mechanism for this reaction is shown in (Scheme 5).
Biological investigation
Cytotoxic activity
The in vitro growth inhibitory rates against human lung carcinoma (A549) cell line and effective antitumor doses (as measured by IC₅₀) of the synthesized compounds are investigated in comparison with the well-known antican-cer standard drugs toxoflavin and 5-fluorouracil, using crystal violet colorimetric viability assay. Data generated
are used to plot dose response curves and presented in Table 1 and Fig. 1. The results reveal that all the tested compounds show high variation in the inhibitory growth rates and activities to the tumor cell line in a concentration dependent manner as shown in (Table 1).

From the results in Fig. 1, it is clear that all the tested compounds are found to be very active at 500 μM against human lung carcinoma (A549) cell line after treatment for 72 h with inhibition ratio values between 60 and 97%. The difference between inhibitory activities of all compounds with different concentrations is statistically significant (p < 0.001).

The highest activity against human lung carcinoma (A549) cell line is measured for compound 6b with IC$_{50}$ value 3.6 μM, followed by compounds 9, 5a, 8, 5e, 6e, 5b, 5f, 7a, 5c, 6c, 7b, 6a, 11, 5d and 6d with IC$_{50}$ values of 26.3, 26.8, 28.4, 49.3, 53.8, 54.7, 60.2, 60.5, 74.3, 81.5, 104.6, 107.1, 123, 238.7, and 379.4 μM, compared with reference drugs 5-fluorouracil (10.5 μM) and toxoflavin (0.7 μM).

Methods

Instruments

All melting points were determined with an electrothermal melting-temperature II apparatus and are uncorrected. Element analyses are performed at the regional

**Table 1** The IC$_{50}$ values represent the compound concentration (μM) required to inhibit A549 tumor cell proliferation by 50%

| Compounds | IC$_{50}$ (μM) | Compounds | IC$_{50}$ (μM) |
|-----------|---------------|-----------|---------------|
| 5a        | 26.8±1.3      | 6c        | 81.5±4.3      |
| 5b        | 54.7±2.1      | 6d        | 379.4±24.8    |
| 5c        | 74.3±5.1      | 6e        | 53.8±3.5      |
| 5d        | 238.7±12.5    | 7a        | 60.5±2.6      |
| 5e        | 49.3±4.1      | 7b        | 104.6±4.8     |
| 5f        | 60.2±3.2      | 8         | 28.4±1.6      |
| 6a        | 107.1±6.2     | 9         | 26.3±0.9      |
| 6b        | 3.6±0.2       | 11        | 123±6.1       |
| Toxoflavin* | 0.7±0.1     | 5-FU (5-fluorouracil) | 10.5±0.1 |

* Reference drugs; 5-FU (5-fluorouracil)
Fig. 1 Growth inhibition curves showing A549 cell line treated with the tested compounds at different concentrations compared with reference drugs 5-flourouracil and toxoflavin
center for mycology and biotechnology at Al-Azhar University. The infrared (IR) spectra are recorded using potassium bromide disc technique on Nikolet IR 200 FT IR. Mass spectra are recorded on a DI-50 unit of Shimadzu GC/MS-QP 5050A at the regional center for mycology and biotechnology at Al-Azhar University. 1H-NMR and 13C-NMR spectra are determined on Bruker 400 MHz spectrometer using DMSO-d$_6$ as a solvent, applied nucleic acid research center, Zagazig University, Egypt. All reactions are monitored by TLC using precoated plastic sheets silica gel (Merck 60 F254). Spots are visualized by irradiation with UV light (254 nm). The used solvent system is chloroform: methanol (9:1) and ethyl acetate: toluene (1:1).

4-Chlorobenzaldehyde(2,6-dioxo-3-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)hydrazone (5c)

A mixture of 6-hydrazinyl-1-propyluracil (5a) and appropriate benzaldehydes (2.17 mmol) in ethanol and crystallized from ethanol.

Benzaldehyde(2,6-dioxo-3-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)hydrazone (5a)

Yield: 83%; m.p. = 218–219 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3224 (NH), 3045 (CH arom.), 2969, 2908 (CH aliph.), 1740, 1647 (C=O), 1512 (C=N), 1506, 1455, 1333, 1319, 1287, 1232, 77.4, 42.2, 21.0, 10.7 ppm; MS: $m/z$ (%): $M^+356$, 272 (83), 243 (61), 216 (36), 153 (36), 145 (31), 144 (25), 110 (27), 106 (58), 104 (100), 103 (22), 90 (38), 89 (33), 77 (52); Anal. calcld. for C$_{14}$H$_{15}$N$_2$O$_2$: C, 56.47; H, 4.81; N, 19.69. Found: C, 56.26; H, 4.83; N, 19.57.

4-Bromobenzaldehyde(2,6-dioxo-3-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)hydrazone (5d)

Yield: 78%; m.p. = 213–214 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3560 (OH), 3271 (NH), 3020 (CH arom.), 2968 (CH aliph.), 1705, 1625 (C=O), 1581 (C=N), 1512 (C=C), 834 (p-substituted phenyl); 1H-NMR (DMSO-d$_6$): 10.58 (s, 1H, NH), 10.15 (s, 1H, OH), 8.28 (s, 1H, CH), 7.56–7.53 (d, 2H, $J$ = 8.4 Hz, H$_{17}$), 6.84–6.82 (d, 2H, $J$ = 8.4 Hz, H$_{16}$), 3.52 (s, 1H, CH), 2.77–2.74 (t, 2H, CH$_2$), 1.59–1.57 (m, 2H, CH$_2$), 0.93–0.91 (s, 3H, CH$_3$); 13C-NMR (DMSO-d$_6$): $\delta$ = 162.5, 159.4, 152.5, 151.1, 147.0, 128.7, 125.1, 115.8, 76.6, 42.1, 21.0, 10.7 ppm; MS: $m/z$ (%): $M^+356$, 288 (40), 259 (21), 232 (31), 161 (72), 160 (48), 146 (24), 122 (61), 121 (26), 120 (88), 119 (100), 106 (30), 1105 (20); Anal. calcld. for C$_{14}$H$_{15}$BrN$_2$O$_2$: C, 58.32; H, 5.59; N, 19.43. Found: C, 58.50; H, 5.01; N, 18.43.

4-Hydroxybenzaldehyde(2,6-dioxo-3-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)hydrazone (5e)

Yield: 91%; m.p. = 228–230 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3150 (NH), 3022 (CH arom.), 2968 (CH aliph.), 1740, 1692 (C=O), 1593 (C=N), 1561 (C=C), 1514 (NO$_2$ asymstr), 1418 (NO$_2$ symstr).
3.87 (t, 2H, CH₂), 1.61–1.55 (m, 2H, CH₂), 0.91–0.87 (t, 3H, CH₃); ³¹C-NMR (DMSO-d₆): δ = 162.4, 152.3, 151.0, 147.7, 143.7, 140.3, 127.8, 124.1, 78.1, 42.3, 21.1, 10.7 ppm; MS: m/z (%) = M⁺, 317 (39), 288 (100), 261 (83), 190 (38), 168 (22), 153 (91), 152 (54), 151 (34), 149 (33), 127 (41), 110 (34), 89 (60), 84 (31), 76 (33), 68 (50); Anal. calcld. for C₁₄H₁₅N₅O₄ (317.30): C, 52.99; H, 4.67; N, 22.49. Found: C, 53.15; H, 4.83; N, 22.24.

4-(Dimethylamino)benzaldehyde (2,6-dioxo-3-propyl-1,2,3,6-tetrahydro-pyrimidin-4-yl)hydrazone (5f)

Yield: 76%; m.p. = 234–235 °C; IR (KBr) ν max (cm⁻¹): 3220 (NH), 3045 (CH arom.), 2968, 2958 (CH aliph.), 1729, 1693 (C=O), 1593 (C=N), 1519 (C=C), 855 (C=N), 1519 (C=C), 1324, 1320, 1293, 1286, 42.9, 20.3, 11.0 ppm; MS: m/z (%) = M⁺, 315 (100), 314 (10), 259 (8), 202 (11), 77 (29), 69 (31), 57 (26); Anal. calcld. for C₁₄H₁₃N₅O₂ (283.28): C, 56.34; H, 4.47; N, 23.40. Found: C, 56.34; H, 4.47; N, 23.62.

3-Aryl-8-propylypyrimido[5,4-e][1,2,4]triazine-5,7-(6H,8H)-diones (6a–e)

A solution of 4-substituted benzaldehyde (2,6-dioxo-3-propyl-1,2,3,6-tetrahydro-pyrimidin-4-yl)hydrazone (5a–e) (0.98 mmol) in glacial acetic acid (4 mL) is treated with sodium nitrite (1.16 mmol) by heating under reflux for 3–4 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL); the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:2) to afford compounds 6a–e.

3-Phenyl-8-propylypyrimido[5,4-e][1,2,4]triazine-5,7-(6H,8H)-dione (6a)

Yield: 71%; m.p. = 290–291 °C; IR (KBr) ν max (cm⁻¹): 3173 (NH), 3024 (CH arom.), 2968, 2840 (CH aliph.), 1716, 1670 (C=O), 1565 (C=N), 1535 (C=C); ¹H-NMR (DMSO-d₆): 12.25 (s, 1H, NH), 8.42–8.40 (d, 2H, J = 5.2 Hz, H₄₃), 7.62–7.61 (m, 3H, arom.), 3.24–3.21 (t, 2H, CH₂), 1.68–1.63 (m, 2H, CH₂), 0.98–0.94 (t, 3H, CH₃); ³¹C-NMR (DMSO-d₆): δ = 160.4, 154.7, 150.8, 149.4, 146.2, 134.2, 131.3, 129.3, 127.1, 42.4, 20.6, 11.0 ppm; MS: m/z (%) = M⁺, 315 (16), 283 (16), 255 (20), 254 (38), 213 (17), 171 (13), 105 (100), 104 (13), 103 (10), 77 (29); Anal. calcld. for C₁₄H₁₁N₃O₃ (299.28): C, 56.18; H, 4.38; N, 23.40. Found: C, 56.34; H, 4.47; N, 23.62.
3-(4-Nitrophenyl)-8-propylpyrimido[5,4-c][1,2,4]triazine-5,6(1H,3H)-dione (6c)
Yield: 70%; m.p. = 267–268 °C; IR (KBr) νmax (cm⁻¹): 3165 (NH), 3067 (CH arom.), 2974, 2811 (CH aliph.), 1720, 1702 (C=O), 1606 (C=N), 1559 (C=C), 1518 (NO₂ asym), 1346 (NO₂ sym), 844 (p-substituted phenyl); ¹H-NMR (DMSO-d₆): δ = 12.31 (s, 1H, NH), 8.66–8.62 (d, 2H, J = 9.2 Hz, Hₐrom), 8.46–8.43 (d, 2H, J = 9.2 Hz, Hₐrom), 4.25–4.21 (t, 2H, CH₂), 1.75–1.70 (m, 2H, CH₂), 0.98–0.94 (t, 3H, CH₃); ¹³C-NMR (DMSO-d₆): δ = 162.0, 156.9, 151.4, 151.2, 148.9, 140.4, 134.2, 128.2, 124.4, 42.8, 20.4, 11.1 ppm; MS: m/z (%) = M⁺, 328 (11), 300 (18), 299 (63), 258 (32), 244 (21), 151 (42), 150 (100), 104 (18), 76 (20), 65 (21), 43 (23); Anal. calcld. for C₁₈H₁₆N₅O₂: C, 51.22; H, 3.68; N, 25.60. Found: C, 51.37; H, 3.65; N, 25.81.

6-[1-Arylethylidene]hydrazino]-1-propylpyrimidine-2,4(1H,3H)-diones (7a, b)
A mixture of 6-hydrazinyl-1-propyluracil (4) (2.72 mmol) and appropriate acetophenones (2.72 mmol) in ethanol (30 mL) is stirred at room temperature for 3–4 h. The formed precipitate is collected by filtration, washed with ethanol and crystallized from ethanol.

6-[1-Phenylethylidene]hydrazino]-1-propylpyrimidine-2,4(1H,3H)-dione (7a)
Yield: 83%; m.p. = 207–208 °C; IR (KBr) νmax (cm⁻¹): 3154 (NH), 3052 (CH arom.), 2963, 2870 (CH aliph.), 1715, 1691 (C=C), 1499 (C=C); ¹H-NMR (DMSO-d₆): 11.06 (s, 1H, NH), 8.96 (s, 1H, NH), 7.90–7.82 (dd, 2H, J = 9.2 Hz, Hₐrom), 7.44–7.43 (m, 3H, arom.), 5.39 (s, 1H, CH₃), 3.92–3.90 (t, 2H, CH₂), 2.37 (s, 3H, α-CH₃), 1.68–1.63 (m, 2H, CH₂), 0.93–0.87 (t, 3H, CH₃); ¹³C-NMR (DMSO-d₆): δ = 166.5, 162.5, 161.1, 151.2, 137.6, 129.6, 128.5, 126.5, 78.8, 43.3, 20.2, 14.3, 11.3 ppm; MS: m/z (%) = M⁺, 328 (44), 271 (44), 257 (21), 167 (36), 159 (64), 158 (64), 144 (45), 131 (40), 124 (20), 120 (100), 118 (48), 104 (72), 103 (38), 96 (21), 78 (30), 77 (96); Anal. calcld. for C₁₈H₁₆N₅O₂: C, 51.22; H, 3.68; N, 25.60. Found: C, 51.37; H, 3.65; N, 19.57.

5-[Dimethylaminomethylene]-1-propylpyrimidine-2,4,6(1H,3H,5H)-trione 6-[1-phenylethylidene]hydrazone (8)
Method A A solution of 6-[1-phenylethylidene] hydrazino]-1-propylpyrimidine-2,4(1H,3H)-dione (7a) (0.7 mmol) in dimethylformamide-dimethylacetal (4 mL) is heated under reflux for 1 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL); the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 8.

Method B A solution of 6-[1-phenylethylidene] hydrazino]-1-propylpyrimidine-2,4(1H,3H)-dione (7a) (0.7 mmol) in dimethylformamide-dimethylacetal (1.5 mL) and DMF (1.5 mL) is heated under reflux for 15 min. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL); the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 8.

5-Methyl-2-(1-phenylvinyl)-7-propyl-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (9)
Method A A solution of 6-[2-(1-phenylethylidene) hydrazino]-1-propyl-pyrimidine-2,4(1H,3H)-dione (7a) (1.05 mmol) in dimethylformamide-dimethyl acetal (1.5 mL) and DMF (1.5 mL) is heated under reflux for 1 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL); the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 9.
Method B A mixture of 6-[2-(1-phenylethylidene) hydrazino]-1-propyl- pyrimidine-2,4(1H,3H)-dione (7a) (1.05 mmol) and dimethylformamide-dimethylacetal (3 mL) is heated under reflux for 12 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL), the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 9.

Method C A mixture of 5-[(dimethylamino)methylene]-1-propylpyrimidine-2,6(1H,3H,5H)-trione 6-[[1-phenylethylidene]hydrazone] (8) (1.17 mmol) and DMF-DMA (3 mL) is heated under reflux for 1 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (15 mL), the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 9.

Yield: method A 82%, method B 74%, method C 92%; m.p. 167–169 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 3095 (CH arom.) 2950, 2875 (CH aliph.), 1760, 1701 (C=O), 1591 (C=N), 1546 (C=C); $^1$H-NMR (DMSO-d_6): 8.60 (s, 1H, CH-3), 7.44–7.36 (m, 5H, arom.), 5.71–5.61 (dd, 2H, =CH$_2$), 3.87–3.83 (t, 2H, CH$_2$), 3.23 (s, 3H, CH$_3$), 1.69–1.64 (m, 2H, CH$_2$), 0.86–0.83 (t, 3H, CH$_3$); $^{13}$C-NMR (DMSO-d$_6$): $\delta$ = 157.8, 151.0, 150.3, 144.6, 134.2, 131.8, 129.5, 128.5, 127.3, 109.0, 101.7, 44.7, 27.6, 20.2, 11.0 ppm; MS: m/z (%) = M$^+$, 310 (100), 268 (75), 267 (54), 224 (65), 122 (64), 103 (67),77 (34); Anal. calc. for C$_{17}$H$_{18}$N$_4$O$_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 66.01; H, 5.89; N, 18.24.

2,5-Dimethyl-7-propyl-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (11)

A solution of 6-hydrazinyl-1-propyluracil (4) (1.63 mmol) in dimethylformamide-dimethylacetal (1.5 mL) and DMF (1.5 mL) is heated under reflux for 1 h. The reaction mixture is evaporated under reduced pressure. The residue is treated with ethanol (10 mL), the formed precipitate is filtered, washed with ethanol, and crystallized from DMF/ethanol (1:3) to afford compound 11.

Yield: 88%; m.p. = 167–169 °C; IR (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 3094 (CH arom.), 2951, 2877 (CH aliph.), 1751, 1698 (C=O), 1586 (C=N), 1544 (C=C); $^1$H-NMR (DMSO-d$_6$): 8.41 (s, 1H, CH-3), 3.88 (s, 1H, N(2)-CH$_3$), 3.87–3.83 (t, 2H, CH$_2$), 3.19 (s, 3H, N(5)-CH$_3$), 1.70–1.65 (m, 2H, CH$_2$), 0.90–0.86 (t, 3H, CH$_3$); $^{13}$C-NMR (DMSO-d$_6$): $\delta$ = 157.9, 151.0, 149.8, 131.8, 100.1, 44.7, 40.4, 27.5, 20.3, 11.0 ppm; MS: m/z (%) = M$^+$, 222 (30), 180 (52), 135 (100), 123 (28), 42 (15); Anal. calc. for C$_{16}$H$_{16}$N$_4$O$_2$ (222.24): C, 54.04; H, 6.35; N, 25.21. Found: C, 54.13; H, 6.43; N, 25.45.

Biological investigation

Evaluation of the antitumor activity

Mammalian cell lines

The cell line that used in this study was human lung carcinoma cell line (A549 cells) is obtained from tissue culture Unit, VACSERA, Cairo, Egypt.

The mammalian cells are propagated in Dulbecco's modified Eagle's [41] medium (DMEM) or RPMI-1640 depending on the type of cell line supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 μg/mL gentamycin. All cells are maintained at 37 °C in a humidified atmosphere with 5% CO$_2$ and are subcultured two times a week along experimentation.

i-Antitumor activity evaluation using viability assay

Antitumor activity assay is carried out according to the method described literature [42]. All the experiments concerning the cytotoxicity evaluation are performed and analyzed by tissue culture unit at the regional center for mycology and biotechnology RCMB, Al-Azhar University, Cairo, Egypt.

Procedure

The A549 tumor cells are seeded in 96-well plate in 100 μL of growth medium at a cell concentration of 1 × 10⁴ cells/well. After 24 h of seeding, the monolayers are then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells are treated with 100 μL from different dilutions of the test sample in fresh maintenance medium and incubated at 37 °C. Different two-fold dilutions of the tested compound (started from 500 to 0.25 μM) are added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates are incubated at 37 °C in a humidified incubator with 5% CO$_2$ for a period of 72 h. Untreated cells are served as controls. Three independent experiments are performed each containing six replicates for each concentration of the tested samples. The cytotoxic effects of the tested compounds are then measured using crystal violet staining viability assay. Briefly, after 72 h of treatment, the medium is removed, 100 μL of 0.5% of crystal violet in 50% methanol is added to each well and incubated for 20 min at room temperature and subsequently excess dye is washed out gently by distilled water. The plate is allowed to dry then the viable crystal violet-stained cells are lysed using 33% glacial acetic acid solution. Absorbance at 570 nm is then measured in each well using microplate reader (Sunrise, TECAN, Inc, USA). Toxoflavin and 5-fluorouracil are used as positive...
control. The absorbance is proportional to the number of surviving cells in the culture plate.

**Conclusions**

A series of newly synthesized compounds of substituted benzaldehyde-pyrimidin-4-yl)hydrazones (5a–f), pyrimido[5,4-e][1,2,4]triazines 6a–e, arylethylenediaminyl-pyrimidinyl-pyrimidines 7a,b and pyrazolopyrimidines 9,11 are prepared via a simple method starting from the substrate 6-hydrazinyl-1-propyluracil (4). The synthesized compounds exhibited good cytotoxic activity against human lung carcinoma (A549) cell line and the highest effect is measured for compound 6b with IC$_{50}$ value 36.3 μM, followed by compounds 9, 5a, 8, 5e, 6e, 5b, 5f, 7a, 5c, 6c, 7b, 6a, 11, 5d and 6d with IC$_{50}$ values of 26.3, 26.8, 28.4, 49.3, 53.8, 54.7, 60.2, 60.5, 74.3, 81.5, 104.6, 107.1, 123, 238.7, and 379.4 μM, compared with reference drug 5-fluorouracil (10.5 μM).

**Authors’ contributions**

SAEK formulated the research idea, conceived and prepared the manuscript; SAEK wrote the paper. The author read and approved the final manuscript.

**Author details**

1. Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Giris), Al-Azhar University, Nasr City, Cairo 11961, Egypt.
2. Department of Medical Chemistry, Faculty of Applied Medical Sciences (Female Section), Jazan University, Jazan 45142, Saudi Arabia.

**Acknowledgements**

The Author wishes to thank Dr. Mahmoud Elasser for carrying out and drafting the biological activity of this work, who is an associate professor at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

**Competing interests**

The author declares no competing interests.

**Ethics approval and consent to participate**

Not applicable.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**References**

1. Izmest’ev AN, Gazeiva GA, Sigay NV, Serkov SA, Karnoukhova VA, Kashala VV, Shashkov AS, Zanin IE, Kravchenko AN, Makhova NN (2016) An effective one-pot access to polymeric dispiroheterocyclic structures comprising pyrrolidinyloxadiazole and imidazoxadiazoline triazines via a 1,3-dipolar cycloaddition strategy, Beilstein J Org Chem 12:2240-2249
2. Ruanapan P, Laatsch H, Tangchitsomkid N, Lumyong S (2011) Nematidical activity of vervulin isolated from a nematicidal actinomycete, Streptomyces sp. CMU-MH021, on Meloidogyne incognita. World J Microbiol Biotechnol 27:1373-1380
3. Nagamatsu T, Tamasaki H, Hirota T, Yamato M, Kido Y, Shibata M, Yoneda F (1993) Syntheses of 3-substituted 1-methyl-6-phenylpyrimido[5,4-e] (1,2,4)triazine-5,7(1H,6H)-diones (6-phenyl analogs of toxoflavin) and their 4-oxides, and evaluation of antimicrobial activity of toxoflavins and their analogs. Chem Pharm Bull 41:362–368
4. Kiselev OI, Deyeva EG, Melnicova TI, Kozatekka KN, Kiselev AS, Rusinov VL, Charsun VN, Chupakhin ON (2012) A new antiviral drug triazavirin: results of phase II clinical trial. Vopr Virusol 57:9–12
5. Abdel-Rahman RM, Seada M, Fawzy M, El-Baz I (1994) Synthesis of some new 1,6-di-hydrazinyl-3-substituted 6-spino-[9f]-fluorene]-1,2,4-triazin-5(4H)-ones as potential anti HIV and antitumor drugs. Pharmazie 49:729–733
6. Abdel-Rahman RM, Seada M, Fawzy M, El-Baz I (1994) Synthesis of some new 1,6-di-hydrazinyl-3-substituted 6-spino-[9f)-fluorene]-1,2,4-triazin-5(4H)-ones as potential anti HIV and antitumor drugs. Bioorg Chem Lett 21:5296–5300
7. Crespin L, Biancalana L, Morack T, Blakemore DC, Ley SV (2017) One-pot acid-catalyzed ring opening/cyclization/oxidation of aziridines with N-tosylhydrazones: access to 1,2,4-triazines. Org Lett 19:1084–1087
8. Sztanke K, Pasternak K, Rajtar B, Sztanke M, Majek M, Polz-Dacewicz M (2007) Identification of antibacterial and antiviral activities of novel fused 1,2,4-triazine esters. Bioorg Med Chem. 15:5480–5486
9. Cukalova H, Dzugosova V, Gbelskya Y, Subik J (2013) Antibacterial activity of CTBT (7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine) generating reactive oxygen species. Microbiol Res 168:147–152
10. Yurttas L, Demirayak S, Ilgin S, Ali O (2014) In vitro antitumor activity evaluation of some 2,4,6-triazine derivatives bearing piperazine amide moiety against breast cancer cells. Bioorg Med Chem 22:6313–6323
11. Kerrizmarzyk Z, Wysoczki W, Urbanzcy-Lipowsa Z, Kalcic P, Belawza A, Belawza K, Laweca J (2015) Synthetic approaches for sulfur derivatives containing 1,2,4-triazine moiety: their activity for in vivo screening towards two human cancer cell lines. Chem Pharm Bull. 63:531–537
12. Ianneja H, Nadri H, Naderi N, Rezaeian SN, Zafari M, Foroumadi A (2015) Anticonvulsant activity of 1,2,4-triazine derivatives with pyridyl side chain: synthesis, biological, and computational study. Med Chem Res 24(6):2505–2513
13. Khothnevizadeh M, Ghaemnejadi MH, Foroumadi A, Mirm RR, Firiizi O, Madadkar-Sobhani A, Edraki N, Parsa M, Shafiee A (2013) Design, synthesis and biological evaluation of novel anti-cytokine 1,2,4-triazine derivatives. Bioorg Med Chem 21:6708–6717
14. Rusinov VL, Ergonov VN, Chupakhin ON, Belanov EF, Bormotov NI, Serova OA (2012) Synthesis and antiviral activity of 1,2,4-triazine derivatives. Pharm Chem J 45:635–669
15. Sidwell RW, Dixon GJ, Selkirk SM, Schabel FM (1968) In vivo antiviral properties of biologically active compounds. Appl Microbiol 16(2):370–392
16. Falke D, Rada B (1970) 6-Azauridine as an inhibitor of the synthesis of guanine. Acta Virol 14(2):115–123
17. Cressley WA, Fink ME, Handschumacher RE, Calabresi P (1963) Clinical and pharmacological studies with 2′,3′,5′-triacetyl-6-azauridine. Cancer Res 23:444–453
25. Ludovic V, Groleau M, Dekimpe V, Deziel E (2007) Burkholderia diversity and versatility: an inventory of the extracellular products. J Microbiol Biotechnol 17(9):1407–1429
26. Goh KC, Wang H, Yu N, Zhou Y, Zheng Y, Lim Z, Sangthongpitak K, Fang L, Du M, Wang X (2004) PLK1 as a potential drug target in cancer therapy. Drug Dev Res 62:349–361
27. Nagamatsu T (2001) Syntheses, transformation, and biological activities of 7-azapteridine antibiotics: toxoflavin, fervenulin, reumycin and their analogs. Recent Res Dev Org Bioorg Chem 4:97–121
28. Aly AA, Gad El-Karim IA (2011) facile synthesis of new pyrazolopyrimidine derivatives of potential biosignificant interest. J Korean Chem Soc 55(5):781–786
29. Tollefson MB, Acker BA, Jacobsen EJ, Hughes RO, Walker JK, Fox DNA, Palmer MJ, Freeman SK, Yu Y, Bond BR (2010) 1-(2-Ethoxyethyl)-1H-pyrazolo[4,3-d]pyrimidines as potent phosphodiesterase 5 (PDE5) inhibitors. Bioorg Med Chem Lett 20(10):3120–3124
30. Kumar P, Joshi VC (2010) Spectral studies and biological activity of novel 1H-1,4-diazepine derivatives. Indian J Chem Sect B 49B(1):84–88
31. Ivachtchenko AV, Dmitriev DE, Golovina ES, Dubrovskaya ES, Kadieva MG, Koryakova AG, Ksyl VM, Mitkin OD, Tkachenko SE, Okun IM, Vorobiov AA (2010) Synthesis of cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-a]pyrimidines and their evaluation as 5-HT6 receptor antagonists. Bioorg Med Chem Lett 20(7):2133–2136
32. Bakavoli M, Bagherzadeh G, Vaseghifar M, Shiri A, Pordeli P, Araghi M (2010) Molecular iodine promoted synthesis of new pyrazolo[3,4-d]pyrimidine derivatives as potential antibacterial agents. Eur J Med Chem 45(2):647–650
33. Curran KJ, Verheijen JC, Kaplan J, Richard DJ, Toral-Baraza L, Hollander J, Lucas J, Ayral-Kalustian S, Yu K, Zask A (2010) Pyrazolopyrimidines as highly potent and selective, ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR) optimization of the 1-substituent. Bioorg Med Chem Lett 20(4):1440–1444
34. Kim I, Song JH, Park CM, Jeong JW, Kim HR, Ha JR, No Z, Hyun YL, Cho YS, Kang NS, Jeon DJ (2010) Design, synthesis, and evaluation of 2-aryl-7-[(3′,4′-dialkoxyphenyl)-pyrazolo[1,5-a]pyrimidines as novel PDE-4 inhibitors. Bioorg Med Chem Lett 20(3):922–926
35. Ali HI, Fujita T, Akaho E, Nagamatsu T (2010) A comparative study of AutoDock and PMF scoring performances, and SAR of 2-substituted pyrazolotriazolopyrimidines and 4-substituted pyrazolopyrimidines as potent xanthine oxidase inhibitors. J Comput Aided Mol Des 24(1):57–75
36. Schenone S, Brullo C, Bruno O, Bondavalli F, Mosti L, Maga G, Crespan E, Carraro F, Manetti F, Tintori C, Botta M (2008) Synthesis, biological evaluation and docking studies of 4-amino substituted 1H-pyrazolo[3,4-d] pyrimidines. Eur J Med Chem 43(12):2665–2676
37. Cresswell RM, Wood HCS (1960) The biosynthesis of pteridines. Part I. The synthesis of riboflavin. J Chem Soc. 4768–4775
38. El-Kalyoubi S, Agili F (2016) A novel synthesis of fused uracils: indenopyrimidopyridazines, pyrimidopyridazines, and pyrazolopyrimidines for antifungal and antitumor evaluation. Molecules. https://doi.org/10.3390/molecules21121714
39. Furukawa Y, Mali Y (1986) 3-Aminopyrazolo[3,4-d]pyrimidine derivatives and production thereof. US Patent 4,603,203
40. Youssif S, Assy M (1996) Fervenulin, 4-deazafervenulin and 5-deazaalloxazine analogues: Synthesis and Antimicrobial activity. J Chem Res 442:2546
41. Halawa AH, Elaasser MM, El Kerdawy AM, Abd El-Hady AM, Ermam HA, El-Agroy AM (2017) Anticancer activities, molecular docking and structure–activity relationship of novel synthesized 4H-chromene, and 5H-chromeno[2,3-d]pyrimidine candidates. Med Chem Res 26(10):2624–2638
42. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63