Synthesis, Cytostatic and Antibacterial Evaluations of Novel 1,2,3-Triazolyl-tagged Pyrimidine and Furo[2,3-d']pyrimidine Derivatives

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INTRODUCTION

Pyrimidines are essential constituents of all cells and thereby one of the most important heterocyclic compounds.[1,2] Furthermore, pyrimidine based heterocycles are of interest as potential bioactive molecules and possess wide spectrum of biological activities such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antitubercular and antimalarial activity.[3–9] Modified pyrimidine nucleosides were among the first chemotherapeutic agents to be introduced into the medical treatment of cancer.[6,7] In particular, a number of pyrimidine derivatives with potent biological properties have been prepared by substitution at the 5-position of the pyrimidine ring.[8,9] Moreover, various five-membered heteroaromatic ring-fused pyrimidines are purinonimetics which were subjected to biological investigations to assess their potential therapeutic usefulness such as anti-inflammatory, antibacterial, anticancer and antiviral agents.[10–15] Thus, furo[2,3-d]pyrimidine attract considerable attention due to their great practical significance through exerting pharmacological potential as antiviral, antimicrobial, and antitumor
agents, and is one of the most recently explored scaffolds to have potential anticancer activity through inhibition of various protein kinases.[16-19] Besides, it was found that some 1,2,3-triazole tethered pyrimidine nucleosides,[20] 1,2,3-triazole pyrimidine nucleoside conjugates with the 1,2,3-triazole as a substituent at the pyrimidine ring,[21] the sugar moiety[22] or sugar mimic[23] were endowed with a pronounced cytostatic activity.[24] In continuation of our efforts towards the hybridisation of pyrimidine and 1,2,3-triazole scaffolds into a single chemical entity,[25] we report here the synthesis and biological investigations of C-5 alkylated pyrimidines and C-6-alkylated furo[2,3-d]pyrimidines containing N-1-substituted 1,2,3-triazole ring. Moreover, the effect of substituents at pyrimidine, furo[2,3-d]pyrimidine and 1,2,3-triazole moieties on biological activities was assessed.

RESULTS AND DISCUSSION

Chemistry

The novel uracil derivatives with lateral alkynyl substituents at C-5 position of pyrimidine and butenyl or propargyl chains at position N-1 (3-6) were synthesized by the N-alkylation reaction of 5-iodouracil with corresponding alkyl halide in the presence of NaH, as a base, followed by Sonogashira cross-coupling reaction of 5-iodouracil containing 1-butenyl (1) or propargyl chain at N-1 (2) with corresponding terminal alkynes in the presence of Pd catalyst (Scheme 1). Furo[2,3-d]pyrimidine derivatives (7 and 8) were obtained by in situ O-heteroaannulation reaction of N-alkyl-S-alkynylpyrimidines (3 and 5) obtained in the Sonogashira reaction (Scheme 1).

1,2,3-Triazole derivatives (9-13) were synthesized by click reaction of the 5-iodo-N-propargyluracil derivative (2) with corresponding azides using microwave irradiation (Scheme 2). Introduction of alkynyl substituents at C-5 of pyrimidine ring by Sonogashira cross-coupling reaction of 9-13 gave 5-alkynylpyrimidines (14-19) and 6-substituted furo[2,3-d]pyrimidines (20-22) with 1,2,3-triazole moiety at N-1 and N-3, respectively. 6-Substituted furo[2,3-d]pyrimidines (20-22) were obtained by in situ 5-endo-dig cyclization of C-5-alkynyl-N-1-(1,2,3-triazolyl) uracil derivatives (16, 17 and 19) using CuI and base (Scheme 2).

Antiproliferative Evaluations

Effect of pyrimidine and furo[2,3-d]pyrimidine derivatives of 1,2,3-triazole were investigated on the growth of human cervix adenocarcinoma (HeLa), colon adenocarcinoma (CaCo-2), chronic myeloid leukemia in blast crisis (K562), Burkitt lymphoma (Raji), and on the normal Madin Darby canine kidney (MDCK I) cells as well (Table 1). It can be observed that, among all evaluated compounds, C-5-p-tolylethenyl pyrimidine derivative 14 with benzyl moiety at N-1 of 1,2,3-triazole ring exhibited moderate cytostatic effect on HeLa cells. Other compounds were deprived of any inhibitory activities against HeLa and CaCo-2 cells. Importantly, the 1,2,3-triazoly-tagged pyrimidine series, compounds 19 bearing 3,5-difluorophenylethynyl at C-5 of pyrimidine and p-(trifluoromethyl)phenyl at 1,2,3-triazole ring showed marked cytostatic activity (IC50 = 8.4 μM) on K562 cells. Furthermore, its furo[2,3-d]pyrimidine structural analog 22 exhibited also significant antitumor activity (IC50 = 7.9 μM) on Raji cells showing the influence of both 3,5-difluorophenyl and p-(trifluoromethyl)phenyl substituents.
on inhibitory activities against leukemia K562 cells. Notably, compounds 19 and 22 were not cytotoxic to evaluated normal kidney (MDCK I) cells. Compound 15 displayed moderate antitumor effects against HeLa and CaCo-2 cells, while compounds 9, 18 and 20 had only marginal activity against these tumor cell lines (Table 1).

**Antibacterial Evaluations**

The in vitro antibacterial activity of novel pyrimidine and furo[2,3-d]pyrimidine derivatives was tested against Gram-positive bacteria including *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis*, vancomycin-resistant *Enterococcus faecium* (VRE) and Gram-negative bacteria including *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25925), *Acinetobacter baumannii* (ATCC 19606), extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* (Table 2). The obtained results were compared with known antibiotic ciprofloxacin (CIP). As displayed in the Table 2, almost all tested compounds did not show antibacterial activities on the growth of evaluated Gram-positive and Gram-negative bacterial strains, except for pyrimidine derivative 14 with p-tolylethynyl substituent at C-5 and benzyl at 1,2,3-triazole which is the most active of all evaluated compounds on the Gram positive bacterial strains *Enterococcus faecalis* (MIC = 8 µg/mL).

![Scheme 2](image)

**Scheme 2.** Synthesis of N-1,1,2,3-triazolyl (9–13), C-5-alkynyl-N-1,1,2,3-triazolyl pyrimidines (14–19), and C-6-alkyl-N-3,1,2,3-triazolylfuro[2,3-d]pyrimidines (20–22). Reagents and conditions: (i) NaN₃ or arylazide (RN₃), Cu, CuSO₄, H₂O : t-BuOH = 1 : 1, DMF, 80 °C, 300 W, 30–60 min.; (ii) terminal alkyne, CuI, (PPh₃)₄Pd, Et₃N or (iso-Pr)₂NH, DMF, r.t., overnight.

**CONCLUSIONS**

Pyrimidine derivatives containing at N-1 buteny substituent (3) and propargyl (4–6) side chain were synthesized by N-alkylation reaction of 5-iodouracil followed by Pd-catalysed Sonogashira cross-coupling reaction of N-alkyl-5-iodouracil derivatives (1 and 2) with corresponding terminal alkynes. 6-Substituted furo[2,3-d]pyrimidine derivatives (7 and 8) were prepared by intramolecular in situ O-hereoannulation ring closure of N-1-alkyl-C-5-alkynylpyrimidine derivatives (3 and 5). Copper(I)-catalysed click reaction of 5-iodo-N-1-propargylpyrimidine (2) with corresponding azides followed by Sonogashira cross-coupling reaction with terminal alkyne gave novel 1,4-disubstituted 1,2,3-triazole tethered 5-alkynylpyrimidine (14–19) and 6-substituted furo[2,3-d]pyrimidines (20–22). In vitro antiproliferative activity of novel compounds evaluated on human cancer cell lines cervix adenocarcinoma (HeLa), colon adenocarcinoma (CaCo-2), chronic myeloid leukemia in blast crisis (K562), Burkitt lymphoma (Raji) revealed that 3,5-difluorophenyl and p-(trifluoromethyl)phenyl substituents in pyrimidine (19) and furo[2,3-d]pyrimidine (22) derivatives had strong impact on inhibitory effects on the growth of K562 (19, IC₅₀ = 8.4 µM) and Raji (22, IC₅₀ = 7.9 µM) tumor cells. Antibacterial evaluations showed that pyrimidine derivative 14 substituted with p-tolylethynyl at C-5 of
**Table 1.** Inhibitory effects of pyrimidine and furo[2,3-$d$]pyrimidine derivatives on the growth of human tumor cell lines HeLa, CaCo-2, Raji and K562 and normal Madin Darby canine kidney (MDCK I) cells as well.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$/μmol dm$^{-3}$ (a)</th>
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<tbody>
<tr>
<td></td>
<td>HeLa</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 100</td>
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<tr>
<td>5</td>
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<td>&gt; 100</td>
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<td>&gt; 100</td>
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<tr>
<td>22</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>5-FU$^d$</td>
<td>8.2</td>
</tr>
</tbody>
</table>

(a) IC$_{50}$ – Compound concentration that inhibited cell growth by 50 %. Exponentially growing cells were treated with substances during 72-h period. Cytotoxicity was analysed using MTT survival assay.

(b) 5-FU – 5-Fluorouracil.

**Table 2.** Inhibitory effects of pyrimidine and furo[2,3-$d$]pyrimidine derivatives on the growth of Gram positive and Gram negative bacterial strains

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC / μg mL$^{-1}$ (a)</th>
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<tbody>
<tr>
<td></td>
<td>Gram-positive bacterial strains</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus ATCC 25923</td>
</tr>
<tr>
<td>4</td>
<td>256</td>
</tr>
<tr>
<td>5</td>
<td>256</td>
</tr>
<tr>
<td>14</td>
<td>&gt; 256</td>
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<td>21</td>
<td>&gt; 256</td>
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<tr>
<td>22</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>CIP$^{d}$</td>
<td>0.125</td>
</tr>
</tbody>
</table>

(a) Minimal inhibitory concentration.

(b) VRE – vancomycin-resistant Enterococcus faecium.

(c) ESBL – extended spectrum β-lactamase-resistant strains;

(d) CIP – ciprofloxacin.
pyrimidine and benzyl at 1,2,3-triazole is the most active of all evaluated compounds on the Gram positive bacterial strains Enterococcus faecalis (MIC 8 μg/mL).

Overall, compounds 19 and 22 are highlighted as promising candidates for further structure optimization and development of a new and more efficient agent for treatment of rapidly progressive hematological malignancies.

EXPERIMENTAL

Materials and General Methods

Commercially available chemicals were purchased from Sigma Aldrich (Germany) and Acros (Belgium) and where used without purification. All solvents used in synthesis were analytical grade purity and dried. Dichloromethane (CH2Cl2) was stored over 4 Å molecular sieves. Methanol (CH3OH) and tert-butanol (t-BuOH) were stored over 3 Å molecular sieves without distillation. Melting points were determined on a Kofler micro hot-stage instrument (Reichert, Wien) and were uncorrected. Precoated Merck silica gel 60 F254 plates were used for thin-layer chromatography and spots were visualized by shortwave UV light (254 nm). Column chromatography was performed on Fluka silica gel (0.063–0.200 mm), with dichloromethane and dichloromethane as mobile phases. Microwave-assisted syntheses were performed in a Milestone microwave oven using glass cuvettes at 80 °C and 300W under the pressure of 1 bar. NMR spectroscopy 1H and 13C NMR spectra were recorded on a Varian Gemini 300 spectrometer (Ruđer Bošković Institute, Zagreb). Samples were measured in DMSO-d6 solutions at 25 °C in 5 mm NMR tubes. 1H and 13C NMR chemical shifts (δ) in ppm were referred to TMS (δ 0.0 ppm). Individual resonances were assigned on the basis of their chemical shifts, signal intensities, multiplicity of resonances and H-H coupling constants. The electron impact mass spectra and the purity of compounds were assessed by using Agilent Technologies 6410 Triple Quad LC/MS instrument equipped with electrospray interface and triplequadrupole analyzer (LC/MS/MS) in positive instrument mode. High performance LC was performed on Agilent 1100 series system with diode array detector (Zorbax C18 reverse-phase analytical column (2.1–30 mm, 3.5 mm). All compounds used for biological evaluation showed >95 % purity in HPLC-MS/MS system.

Synthesis

Compounds 2, 10–12 and 15 were prepared in accord with modified previously reported procedure.[25] 1-(4-Bromo-2-butenyl)-5-iodomypyrimidin-2,4-dione (1)

Suspension of 5-iodouracil (5-1U) (1.02 g, 4.3 mmol) and sodium hydride (NaH) (98.9 mg, 4.3 mmol) in dimethylformamide (DMF) (40 mL) was stirred at room temperature for 30 minutes. 1,4-Dibromo-2-butene was added (474.8 mg, 3.6 mmol) to the reaction mixture and was stirred at room temperature overnight. Crude product mixture was obtained by removal of the solvent under reduced pressure and washed with ethyl acetate and solution of ammonium chloride (NH4Cl) in water. Organic layer was dried over MgSO4 and concentrated under reduced pressure. Crude product mixture was purified by silica gel column chromatography (chloromethane, then dichloromethane: methanol = 30 : 1) and compound 1 (295 mg, 18.5 %) was isolated as yellow oil. 1H NMR (DMSO-d6): δ 11.64 (1 H, s, NH), 8.14 (1H, s, H-6), 5.88 (2H, dd, J = 4.7 Hz, H-2', H-3'), 4.31 (2H, d, J = 3.8 Hz, H-4'), 4.13 (2H, dd, J = 4.3 Hz, H-1') ppm. 13C NMR (DMSO-d6): δ 158.82 (C-4'), 149.96 (C-2), 147.77 (C-6), 133.62 (C-2'), 126.54 (C-3'), 70.61 (C-5), 48.26 (C-1'), 33.92 (C-4'). MS (ESI): m/z = 370.9 ([M + H]+). Anal. calcd. for C17H15BrN2O2: C, 54.11; H, 3.48; N, 12.85. Found: C, 54.84; H, 3.46; N, 12.91. Relative intensities, multiplicity of resonances and H-H coupling constants.

A. General Procedure for Sonogashira Cross-coupling Reaction

Reaction mixture of compounds 1 or 2 with terminal alkene (1.0–4.2 eq.), tetrakis(triphenylphosphine)palladium(0) (PPh3)2Pd (0.1 eq.), Cu (0.2 eq.) and triethyamin (Et3N) or diisopropylamine ((iso-Pr)2NH) (2 eq.) in DMF (5-20 mL) was stirred under argon or nitrogen atmosphere at room temperature overnight. Removal of the solvent under reduced pressure obtained crude product mixture that was purified by silica gel column chromatography (chloromethane, dichloromethane, dichloromethane: methanol = 200 : 1, 150 : 1, 100 : 1) which resulted in isolation of products 3–8.

(trans)-1-(5-bromo-2-butenyl)-2-(5-(2-(4-methylphenyl) ethynyl)pyrimidin-2,4-dione (3) and (trans)-3-(4-bromo-2-butenyl-1-yl)-6-(4-methylphenyl)furo[2,3-c]pyrimidin-2-one (7)

According to procedure A, solution of compound 1 (230 mg, 0.6 mmol), p-tolylacetylene (0.09 mL, 0.75 mmol), PPh3Pd (87.4 mg, 0.67 mmol), Cu (28.7 mg, 0.15 mmol) and Et3N (0.2 mL, 1.51 mmol) in DMF (10 mL) under Ar atmosphere gave crude product which was purified by silica gel column chromatography with dichloromethane as an eluent to afford transparent oil of compound 3 (48 mg, 21.6 %) and white powder of 7 (109.2 mg, 49.1 %, m.p. 146–148 °C). 3: 1H NMR (DMSO-d6): δ 11.66 (1H, s, NH), 8.41 (1H, s H-6), 7.51 (4H, m, Ph), 5.75 (2H, m, H-2', H-3'), 4.80 (2H, m, H-4'), 4.34 (2H, m, H-1'), 2.42 (3H, s, CH3-Pd) ppm. 13C NMR (DMSO-d6): δ 158.80 (C-4), 149.92 (C-2), 147.80 (C-6), 136.93 (C-6'), 133.58 (C-2'), 131.76 (C-4'), 127.80 (C-5'), 127.06 (C-3'), 119.55 (C-3''), 98.57 (C-5), 91.88 (C-2''), 85.12 (C-1''), 48.26 (C-1'), 33.92 (C-4'), 25.11 (CH3-Pd). MS (ESI): m/z = 360.9 ([M + H]+). Anal. calcd. for C17H13BrN2O2:...
C, 56.84; H, 4.20; N, 7.80. Found: C, 57.08; H, 4.21; N, 7.80. Found: C, 56.71; H, 4.20; N, 7.78.

5-(Decyn-1-yl)-1-(2-propyn-1-yl)pyrimidin-2,4-dione (4)

According to procedure A, solution of compound 2 (200 mg, 0.72 mmol), 1-ethynyl-4-pentylbenzene (0.14 mL, 0.72 mmol), (PPh₃)₂Pd (174.5 mg, 0.07 mmol), CuI (57.4 mg, 0.14 mmol) and Et₃N (0.42 mL, 1.44 mmol) in DMF (10 mL) under N₂ atmosphere gave crude reaction product which was purified by silica gel column chromatography (dichloromethane : methanol = 150 : 1) and white powder of compound 4 (100 mg, 48.4 %, m.p. > 200 °C) was isolated.

1H NMR (DMSO-d₆): δ 11.40 (1H, s, NH), 8.26 (1H, s, H-6), 4.53 (2H, d, J = 2.5 Hz, H-1'), 3.41 (1H, t, J = 2.5 Hz, H-3'), 2.25 (2H, t, J = 6.8 Hz, H-3), 1.50 (2H, m, H-4'), 1.26 (10H, m, H-5''-H-9'), 0.72 (3H, m, C-10') ppm. 13C NMR (DMSO-d₆): δ 159.49 (C-4'), 148.37 (C-2'), 143.50 (C-5), 90.94 (C-2'), 78.42 (C-4'), 75.52 (C-1'), 70.86 (C-3'), 98.90 (C-5), 34.22 (C-1'), 31.48 (C-3'), 28.44, 27.69, 27.25, 27.10, 26.82 (C-4''-C-8''), 23.07 (C-9'), 15.92 (C-10') ppm. MS (ESI): m/z = 287.2 ([M + H]^+). Anal. calcld. for C₁₇H₁₈N₂O₂: C, 71.30; H, 6.80; N, 7.75. Found: C, 71.12; H, 7.75; N, 9.81.

5-((Dodecyn-1-yl)-1-(2-propyn-1-yl)pyrimidin-2,4-dione (5) and 6-decyl-3-(2-propyn-1-yl)uracil[(2,3-dipry) (8)]

According to procedure A, solution of compound 2 (170 mg, 0.66 mmol), dodec-1-ynyl solution of Cu(0) (1.0–1.4 eq.), solution of copper-sulfate (CuSO₄ 1M) (0.1 mL) solution of tert-butanol and water in 1 : 1 ratio (t-BuOH : H₂O = 1 : 1) (10 mL) and dimethylformamide (DMF) (7 mL) was stirred in microwave oven at 80 °C and 300 W for 30–45 minutes. Crude product mixture was removed by the solvent under reduced pressure and was purified by silica gel column chromatography (dichloromethane : methanol = 50 : 1 or 30 : 1) and products 9–13 were obtained.

5-ido-1-(1,2,3-triazol-4-yl)methylpyrimidin-2,4-dione (9)

According to procedure B, solution of compound 2 (100 mg, 0.37 mmol), sodium azide (NaN₃) (28.8 mg, 0.44 mmol), Cu(0) (22.8 mg, 0.037 mmol), CuSO₄ (0.1 mL) and t-BuOH : H₂O = 1 : 1 (10 mL) after evaporation of solvents gave crude product mixture that was purified by silica gel column chromatography (dichloromethane : methanol = 50 : 1) and white crystals of compound 9 (20.2 mg, 16.9 %, m.p. 185–186 °C). 1H NMR (DMSO-d₆): δ 11.74 (1H, s, NH), 8.64 (1H, s, H-3'), 8.23 (1H, s, NH), 8.10 (1H, s, H-6), 4.49 (2H, d, J = 7.2 Hz, H-1') ppm. 13C-NMR (DMSO-d₆): δ 162.06 (C-4'), 151.11 (C-2'), 149.20 (C-6), 143.77 (C-2'), 121.49 (C-3'), 68.51 (C-5), 43.21 (C-1') ppm. MS (ESI): m/z = 319.9 ([M + H]^+). Anal. calcld. for C₁₉H₁₁N₃O₂: C, 26.35; H, 1.90; N, 21.95. Found: C, 26.24; H, 1.91; N, 21.98.
5-iodo-1-{(1-(4-(trifluoromethyl)phenyl)-1,2,3-triazol-4-yl) methyl}pyrimidin-2,4-dione (13)

According to procedure B, solution of compound 2 (200 mg, 0.73 mmol), 1-azido-4-(trifluoromethyl)benzene (1.74 mL, 0.78 mmol), Cu (0) (46.6 mg, 0.73 mmol), 1M CuSO₄ (0.1 mL), t-BuOH : H₂O = 1 : 1 (10 mL) and DMF (7 mL) after evaporation of solvents gave crude product mixture that was purified by silica gel column chromatography (dichloromethane : methanol = 50 : 1) and gave white crystals of compound 13 (195.8 mg, 57.8 %, m.p. 256-258°C). ¹H NMR (DMSO-d₆): δ 11.72 (1H, s, NH), 8.94 (1H, s, H-3'), 8.36 (1H, s, H-6), 8.16 (2H, d, J = 8.5 Hz, H-5'-Ph), 7.99 (2H, d, J = 8.6 Hz, H-4'-Ph), 5.07 (2H, s, H-1''). ¹³C-NMR (DMSO-d₆): δ 161.60 (C-4), 151.89 (C-2), 150.43 (C-7', q, J = 20.2 Hz, C-F), 150.22 (C-6), 144.17 (C-2'), 128.65 (C-4'), 123.76 (CF₃, q, J = 254.2 Hz, C-F), 123.07 (C-5', q, J = 6.6 Hz, C-F), 122.54 (C-3'), 119.28 (C-6', q, J = 8.4 Hz, C-F), 70.82 (C-5), 43.25 (C-1'') ppm. MS (ESI): m/z = 464.2 ([M + H])⁺. Anal. calcld. for C₁₉H₁₇F₃IN₅O₂: C, 63.11; H, 4.98; N, 15.72. Found C, 63.28; H, 1.97; N, 15.11.

1-(4-(1-Benzyl-1,2,3-triazol-4-yl)methyl)-5-(4-tolylethynyl) pyrimidin-2,4-dione (14)

According to procedure A solution of compound 10 (17.8 mg, 0.06 mmol), p-tolylacetylene (34 µL, 0.06 mmol), Cu (0) (3.6 mg, 0.06 mmol), 1M CuSO₄ (0.1 µL), t-BuOH : H₂O = 1 : 1 (1 mL) and DMF (1 mL) after evaporation of solvents gave crude product mixture that was purified by silica gel column chromatography (dichloromethane) and gave yellow crystals of compound 14 (15.1 mg, 63.4 %; m.p. 81-83°C). ¹H NMR (DMSO-d₆): δ 11.63 (1H, s, NH), 8.77 (1H, s, H-6), 7.97 (1H, s, H-3'), 7.61-7.37 (9H, m, Ph), 5.08 (2H, s, CH₂-Pt), 4.61 (2H, s, H-1'), 4.85 (3H, s, CH₃-Pt) ppm. ¹³C-NMR (DMSO-d₆): δ 161.58 (C-4), 152.14 (C-2), 150.09 (C-6), 142.50 (C-2'), 138.52 (C-6'), 136.11 (C-5'), 131.79 (C-4'), 130.18 (C-5''), 128.73 (C-6'), 128.36 (C-3'), 127.37 (C-7'), 120.26 (C-3''), 93.94 (C-5'), 92.66 (C-2'''), 75.28 (C-1'''), 54.24 (C-4'), 42.73 (C-21'), 23.12 (CH₃-Pt) ppm. MS (ESI): m/z = 397.2 ([M+H]⁺). Anal. calcld. for C₁₉H₁₅F₃IN₅O₂: C, 69.51; H, 4.82; N, 17.62. Found C, 69.45; H, 4.81; N, 17.64.

1-(1-(4-(Chlorophenyl)-1,2,3-triazol-4-yl)methyl)-5-(4-tolylethynyl) pyrimidin-2,4-dione (17) and 3-((1-(4-chlorophenyl)-1,2,3-triazol-4-yl)methyl)-6-tolylfuro[2,3-d]pyrimidine (21)

According to procedure A, solution of compound 12 (30 mg, 0.07 mmol), p-tolylacetylene (13 µL, 0.01 mmol), (PPh₃)₂Pt (8 mg, 0.007 mmol), Cu (2.6 mg, 0.014 mmol) and Et₂N (19 µL, 0.14 mmol) in DMF (5 mL) gave crude reaction product that was purified by silica gel column chromatography (dichloromethane, dichloromethane : methanol = 100 : 1) which resulted in isolation of yellow oil of compound 17 (15.1 mg, 51.5 %) and yellow oil of compound 21 (12 mg, 40.9 %). ¹H NMR (DMSO-d₆): δ 11.80 (1H, s, NH), 8.25 (1H, s, H-6), 7.91 (1H, s, H-3'), 7.41 (2H, dd, J = 29.8, 8.1 Hz, Ph), 7.35-7.21 (6H, m, Ph), 4.77 (2H, s, H-1'), 2.33 (3H, s, CH₃-Pt) ppm. ¹³C-NMR (DMSO-d₆): δ 162.26 (C-4), 157.44 (C-7'), 149.48 (C-2'), 147.50 (C-6'), 144.17 (C-2'), 138.43 (C-6''), 133.62 (C-4'), 131.24 (C-4''), 129.40 (C-5''), 123.08 (C-5'), 121.70 (C-3'), 120.35 (C-6'), 119.44 (C-3''), 97.62 (C-5), 93.40 (C-2'''), 73.17 (C-1''), 21.56 (CH₃-Pt) ppm. MS (ESI): m/z = 418.1 ([M+H]⁺). Anal. calcld. for C₁₉H₁₄ClN₃O₂: C, 63.24; H, 3.86; N, 16.76. Found C, 63.33; H, 3.87; N, 16.74. ¹H NMR (DMSO-d₆): δ 8.54 (1H, s, H-4'), 8.05 (1H, s, H-3'), 7.37-7.19 (8H, m, Ph), 6.43 (1H, s, H-5), 4.46 (2H, s, H-1'), 2.31 (3H, s, CH₃-Pt) ppm. ¹³C-NMR (DMSO-d₆): δ 169.25 (C-6), 162.30 (C-7'), 160.81 (C-7a), 154.54 (C-2'), 143.88 (C-2'), 143.30 (C-4), 141.15 (C-3'), 133.49 (C-4'), 131.00 (C-2''), 129.32 (C-3''), 122.80 (C-5''), 121.76 (C-3'), DOI: 10.5562/ccaa3165

120.37 (C-1’), 118.34 (C-6’), 107.55 (C-4a), 98.43 (C-5), 46.40 (C-1’), 19.96 (CH₃) ppm. MS [ESI]: m/z = 417.8 [(M+H)]. Anal. calcd. for C₂₃H₂₄Cl₂O₅: C, 63.24; H, 3.86; N, 16.76. Found C, 63.31; H, 3.85; N, 16.78.

5-(4-Tolyethenyl)-1-((4-(trifluoromethyl)phenyl)-1,2,3-triazol-4-yl)methyl)pyrimidine-2,4-dione (18)

According to procedure A, solution of compound 13 (30 mg, 0.06 mmol), p-toluenesulfonic acid (0.03 mL, 0.25 mmol), (PPh₃)₂Pd (7.4 mg, 0.006 mmol), Cul (2.4 mg, 0.012 mmol) and Et₂N (0.02 mL, 0.12 mmol) in DMF (2 mL) gave crude reaction product that was purified by silica gel column chromatography (dichloromethane) which resulted in isolation of white crystals of compound 18 (13.3 mg, 49.1 %). ¹H NMR (DMSO-d₆): δ 1.17 (4H, s, NH), 8.99 (1H, s, H-3’), 8.24 (1H, s, H-5’), 7.99 (1H, d, J = 8.5 Hz, H-1’), 7.63-7.54 (4H, m, H-1’, H-2’), 7.36 (1H, d, J = 8.5 Hz, H-4’), 7.22 (1H, d, J = 7.9 Hz, H-2’), 5.12 (2H, s, H-1’), 2.33 (3H, s, CH₃) ppm. ¹³C-NMR (DMSO-d₆): 162.49 (C-1’), 151.87 (C-2’), 150.58 (C-7’, q, J = 24.1 Hz, C-F), 149.64 (C-6), 141.73 (C-12’), 138.50 (C-6’), 132.01 (C-1’), 128.65 (C-3’), 128.40 (C-5’), 123.97 (C-5’, q, J = 2.3 Hz, C-F), 123.85 (CF₃, q, J = 251.8 Hz, C-F), 123.72 (C-3’), 120.41 (C-3’), 119.44 (C-6’, q, J = 8.1 Hz, C-F), 94.48 (C-5), 93.17 (C-2’), 75.26 (C-1’), 48.38 (C-1’), 23.10 (CH₃) ppm. MS (ESI): m/z = 452.4 [(M + H⁺)]. Anal. calcd. for C₂₉H₂₃F₂N₂O₂: C, 59.92; H, 2.56; N, 14.80. Found C, 60.69; H, 2.57; N, 14.78.

Biological Evaluations

Cell Cultured

Cells were cultured in tissue culture flasks (25, 75 cm²) in humidified atmosphere under the conditions of 37 °C / 5 % of CO₂ gas in the CO₂ incubator (ISO 150 CELLITime™, JOUAna, Thermo Fisher Scientific, Waltham, MA, USA). HeLa, CaCo-2 and MDCK I were maintained in DMEM medium complemented with 10 % heat-inactivated FBS, 2 mM glutamine, and 100U / 0.1 mg penicillin / streptomycin. Cell lines in suspension, K562 and Raji, were cultured in RPMI-1640 medium complemented with 10 % heat-inactivated FBS, 2 mM glutamine, 1 mM Na-pyruvate, and 10 mM HEPEs. Cell viability was assessed by the trypan blue dye exclusion method before each experiment.

Cytotoxicity evaluation

Cytotoxic effects on the tumors cell growth were determined using the colorimetric methylene blue (MTT) assay. Experiments were carried out on four tumor human cell lines (HeLa, CaCo-2, K562 and Raji) and on one canine cell line (MDCK I) as normal cells. The adherent cells were seeded in 96 micro-well plates at a concentration of 2 x 10⁴ cells/mL and allowed to attach wall plate overnight in the CO₂ incubator. After 72 hours of incubation with tested compounds, the medium was replaced with 5 mg/mL MTT solution and the resulting formazane crystals were dissolved in DMSO. Suspension cells (K562 and Raji) at a concentration of 1 x 10⁵ cells/mL, were plated onto 96 micro-well plates and the same day were treated with tested extracts at different concentrations. After expired 72 hours of incubation, 1 mg/mL MTT solution was added to each well and incubated 4 hours in CO₂ incubator. To each well, 10 % SDS with 0.01 ml/L HCl was added to dissolve water-insoluble MTT-formazane crystals overnight. Elisa micro plate reader (iMark, BIO RAD, Hercules, CA, USA) was used for measurement of absorbance at 595 nm.

All experiments were performed at least three times in triplicates. The percentage of cell growth (PG) was calculated using the following equation:

\[ PG = \frac{A_{\text{compound}} - A_{\text{background}}}{A_{\text{control}} - A_{\text{background}}} \times 100 \]
where $A_{\text{background}}$ at the adherent cells is absorbance of MTT solution and DMSO; $A_{\text{background}}$ at cells growing in suspension is absorbance of the medium without cells, but containing MTT and 10% SDS and 0.01 mol/L HCl; and $A_{\text{control}}$ is the absorbance of cell suspension grown without tested compounds.

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