ANTITUMOR ACTIVITY OF SOME NEW 1,3,8-TRISUBSTITUTED PURINE-2,6-DIONES AND 1,3,6-TRISUBSTITUTED THIAZOLO[2,3-f]PURINE-2,4-DIONES

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New 1,3,8-trisubstituted purine-2,6-diones and 1,3,6-trisubstituted thiazolo[2,3-f]purine-2,4-diones were designed and synthesized as potential antitumor agents. The cytotoxic effects of the tested compounds were assessed against two human malignant cell lines: T-cell leukemia derived SKW-3 and breast cancer – derived MDA-MB-231 using the methyl thiazolyl tetrazolium (MTT-dye) reduction assay, after 72 h exposure. The data were fitted to sigmoidal concentration response curves and the corresponding IC₅₀ values were calculated using commercially available software (GraphPad Prizm). Compound AH-206 was the most potent cytotoxic agent among the newly synthesized compounds, with IC₅₀ value of 17.3 µM. Prominent activity was also encountered with compounds AH-201, AH-205, AH-208, AH-214 and AH-217, all having IC₅₀ values below 100 µM.
INTRODUCTION

The pyrimidine and purine ring systems undoubtedly belong to the most ubiquitous heterocycles in nature, as they represent the main structure of many biologically significant compounds. Several of these heterocycles have been claimed to possess a multitude of pronounced biological activities. The class of fused purines are considered to be attractive targets since its fundamental skeleton is analogues to naturally occurring purine alkaloids. Many biological activities have been reported for these classes of compounds such as phosphodiesterase inhibitors, antimicrobial, anti-asthmatic, antiinflammatory agents, antidiabetic through inhibition of phosphoenolpyruvate carboxykinase (PEPCK). Recently, we reported 1,8-disubstituted purine-2,6-diones as potent analgesic and anti-inflammatory agents through adenosine receptor antagonism and also 3,6-disubstituted thiazolo[2,3-f]purine-2,4-diones as potent analgesic and anti-inflammatory. Caffeine and xanthine derivatives increase the concentration of doxorubicin concentration in Ehrlich ascites carcinoma cells and P388 leukemia cells, through suppression of the doxorubicin efflux from cells in-vitro. Moreover purine derivatives exhibited significant cytotoxic activity against several cancer cell lines. Also, purinoquinazoline derivatives retained a significant cytotoxic activity against the solid tumor (HeLa) and promyelotic human leukaemia (HL60) cell lines through DNA binding and inhibition of topoisomerase II. These facts motivated our interest in the present investigation towards the design and synthesis of new 1,3,8-trisubstituted purine-2,6-diones and 1,3,6-trisubstituted thiazolo[2,3-f]purine-2,4-diones as potential antitumor agents.

EXPERIMENTAL

Materials and equipments

The Melting points were measured using a Stuart Scientific melting point apparatus SMP3 (U.K.) and are uncorrected. The NMR spectra were taken using Bruker DPX 500 MHz instrument at Organic Chemistry institute, Bonn, Germany. DMSO-d6 was used as solvent and the chemical shifts are given in δ (ppm) values. The chemical shifts of the remaining protons of the deuterated solvent served as internal standard: δ 1H: 2.49 ppm, 13C: 39.7 ppm. The EI-MS was obtained using EI- Finnigan MAT 95XL (Thermo Finnigan, Bremen) and FAB-MS was obtained using Concept 1H (Kratos, Hofheim), with m-Nitrobenzyl alcohol as matrix at Organic Chemistry institute, Bonn, Germany. Elemental microanalyses were performed using VarioEL apparatus at Pharmaceutical Institute, Bonn-Endenich, Germany. Silica gel column chromatography was carried out using kieselgel 60 (merck). TLC analysis was performed on kieselgel 60 F254 (Merck) aluminum plates.
Compounds AH-201 - AH-214, 6-amino-3-(2-fluorobenzyl)-1H-pyrimidine-2,4-dione 1, 6-amino-3-(2-fluorobenzyl)-5-nitroso-1H-pyrimidine-2,4-dione 2 and 5,6-diamino-3-(2-fluorobenzyl)-1H-pyrimidine-2,4-dione 3 were prepared as we reported in a recent pervious paper.  

Chemistry  

1-(2-Fluorobenzyl)-8-thioxo-3,7,8,9-tetrahydropurine-2,6-dione (4) (AH-216) Potassium hydroxide (1.5 g, 27 mmol) was dissolved in 40 mL ethanol then carbon disulfide (2.05 g, 27 mmol) was added followed by addition of 5,6-diamino-3-(2-fluorobenzyl)uracil (6.8 g, 27 mmol) 3. The reaction mixture was refluxed for 5 h, diluted with warm water (30 mL) and stirred well, then acetic acid (3 mL) in water (5 mL) was added portionwise. The reaction mixture was allowed to cool in refrigerator for 3 h, the product was collected by filtration. Recrystallization from ethanol to afford the compound 4 (7 g, 88%) as white color crystals: mp 298-300°C (Scheme 1).  

1H-NMR, (500 MHz, DMSO-d6) δ 5 (s, 2H, CH), 7.1-7.3 (m, 3H, phenyl CH), 7.3-7.4 (m, 1H, phenyl CH), 12.9 (br s, 3H, N3-H, N7-H and N9-H).  

13C-NMR: (125 MHz, DMSO-d6), δ 37.5, 103.8, 115.6, 124.7, 129.2, 138.7, 150.4, 152.3, 159.1, 161.5, 164.7, 210.5.  

Anal. Calcd. for C_{13}H_{10}FNO_{2}S: C, 49.31; H, 3.10; N, 19.17. Found: C, 49.53; H, 3.19; N, 19.36.  

FAB-MS, m/z (relative intensity): 293 [(M+1)^{+}, 100%], 292 [(M)^{+}, 30%], 273 [10%], 259 [16%].  

[1-(2-fluorobenzyl)-2,6-dioxo-3,7,8,9-tetrahydro-1H-purin-8-ylsulfonyl]-acetic acid ethyl ester (5) (AH-217). To a solution of 1-(2-Fluorobenzyl)-8-thioxo-3,7,8,9-tetrahydropurine-2,6-dione (0.85 g, 2.9 mmol) 4, dissolved in aqueous sodium hydroxide (1%, 20 mL) was added portionwise with stirring a solution of the ethylbromoacetate (0.48 g, 2.9 mmol) in ethanol (5 mL). The reaction mixture was stirred at ambient temperature for 8 h. The reaction mixture was kept at room temperature overnight and the product was collected by filtration, washed with water and crystallized from ethanol to afford the compounds 5 (0.92 g, 84%) in white color: mp 268-269°C (Scheme 1).  

1H-NMR, (500 MHz, DMSO-d6) δ 1.2 (t, 3H, J= 7.3 Hz, CH₃), 4.1-4.2 (m, 4H, CH₂ & S-CH₂), 5 (s, 2H, CH₂), 7.0-7.2 (m, 3H, phenyl CH), 7.2-7.3 (m, 1H, phenyl CH), 12.0 (br s, 1H, N3-H), 13.5 (br s, 1H, N3-H).  

13C-NMR: (125 MHz, DMSO-d6), δ 14.4, 33.9, 37.5, 61.7, 108.2, 115.6, 124.7, 128.2, 129.1, 129.2, 148.3, 151.1, 154.2, 158.6, 161.9, 168.7. HRMS (EI): calcd. for C_{16}H_{13}FN_{2}O_{2}S: 378.0798, found 378.0803.  

Antitumor activity  

The antitumor activity was carried out at the Department of Pharmacology and Toxicology Faculty of Pharmacy, Sofia, Bulgaria.
Scheme 1: Synthesis of compounds 4 (AH-216) and 5 (AH-217), reagents and conditions: (A) HMDS, (NH₄)₂SO₄, 2-fluorobenzylbromide, Na₂S₂O₃, NaHCO₃, reflux, (B) NaNO₂, 50% CH₃COOH, heat 70°C, 30 min., (C) Na₂S₂O₄, 12.5% NH₃, heat at 60-70°C, 20 min., (D) ethanol, KOH, CS₂, reflux 5 h, (E) 1% NaOH, ethanol, ethylbromoacetate, stirring, 8 h.

Drugs, Chemicals and reagents
The referent anticancer drug cisplatin was purchased from Sigma and used as positive control. Stock solutions of all agents under investigation were freshly prepared in DMSO and promptly diluted serially with RPMI-1640 medium to the desired extend. At the final dilutions obtained the concentration of the solvent never exceeded 1%.

Growth media, RPMI-1640, Dulbecco’s Modified Eagle’s Medium (DMEM), fetal calf serum and L-glutamine were purchased from Sigma. (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)- Triton X-100, and DMSO were supplied form Merck Co.

Cell lines and culture conditions
The T-cell leukemia SKW-3 and the breast cancer MDA-MB-231 were
obtained from DSMZ GmbH (Braunschweig, Germany). SKW-3 cells were maintained as suspension type cultures and MDA-MB-231 as adherent cultures in a controlled environment: RPMI-1640 medium, supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine, at 37°C in a ‘Heraeus’ incubator with 5% CO₂ humidified atmosphere. In order to keep cells in log phase the cultures were refed with fresh RPMI-1640 medium two or three times/week.

Cytotoxicity assay

Cell viability was assessed using the standard MTT-dye reduction assay as previously described with minor modifications. Exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μl/well) at a density of 1x10^5 cells per ml and after 24 h incubation at 37°C they were exposed to various concentrations of the tested compounds for 72 h. For each concentration at least 8 wells were used. After the incubation with the test compounds MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide- Sigma) solution (10 mg/ml in PBS) was added (10 μl/well). Microplates were further incubated for 4 h at 37°C and the quantity of formazan product obtained was determined spectrophotometrically using a microprocessor controlled multiplate reader (Labexim-LMR1) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control (untreated control = 100%). In addition IC₅₀ values were derived from the concentration-response curves. All tests were run in triplicate with cisplatin used as referent positive control.

RESULTS AND DISCUSSION

Chemistry

The synthesis of 6-Amino-3-(2-fluorobenzyl)-5-nitroso-1H-pyrimidine-2,4-dione 2 were prepared as we reported (Scheme 1). 6-Amino-3-(2-fluorobenzyl)-1H-pyrimidine-2,4-dione 1, was prepared by regioselective alkylation of 6-aminouracil in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) as we reported. Then followed by nitrosation and reduction as described to obtain (2) and (3) respectively using reported procedure for similar derivatives.

1-(2-Fluorobenzyl)-8-thioxo-3,7,8,9-tetrahydropurine-2,6-dione (4) was synthesized by reaction of 5,6-diamino-3-(2-fluorobenzyl)-1H-pyrimidine-2,4-dione 3 with carbon disulfide in the presence of potassium hydroxide with slightly modification. Their structures were verified by ¹H-NMR, ¹³C-NMR, elemental analysis and FAB-Ms as illustrated above. The compounds 4 was subjected to the interaction with the ethylbromo actate in the presence of potassium hydroxide to give the required [1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl-sulfonyl]acetic acid ethyl ester (5). The structure elucidation of this newly synthesized derivative was
confirmed by HRMS and spectral data. The NMR spectra of compound 5 are characterized by appearance of the methylene protons of S-CH$_2$ at δ 4.2 and 61.7 ppm in $^1$H-NMR and $^{13}$C-NMR respectively. In addition, the introduced ethyl moiety and the presence of N1-H and N7-H signals is a strong support for an S-alkylation reaction rather than N-alkylation. It is well known that mercaptopurines undergo S-alkylation at lowered temperature while at elevated one N7-H is also attacked.$^{25}$

**Antituomr activity**

The cytotoxic effects of the tested compounds (Scheme 2) were assessed against two human malignant cell lines T-cell leukemia derived SKW-3 and breast cancer derived MDA-MB-231 using the MTT-dye reduction assay, after 72 h exposure. The data were fitted to sigmoidal concentration response curves and the corresponding IC$_{50}$ values were calculated using commercially available software (GraphPad Prizm).

As evident from the results obtained (Table 1) the leukemic cell line was more responsive to the cytotoxic effects of the tested agents and actually all of the reached 50% inhibitory effects upon cellular survival and proliferation. Compound AH-206 was the most potent cytotoxic agent among the newly synthesized compounds, with an IC$_{50}$ value of 17.3 µM. Prominent activity was also encountered with compounds AH-201, AH=205, AH-208, AH-214 and AH-217, all having IC$_{50}$ values below 100 µM. The other compounds were far less active against the T-cell leukemia SKW-3.

The other tumor model – the breast carcinoma-derived MDA-MB-231 cell line was less sensitive than SKW-3 and actually was found to be refractory to some of the compounds, which failed to induce 50% inhibition of cellular proliferation within the concentration range under evaluation (25-400 µM). Superior activity among the novel compounds was established with analogues AH-206 and AH-208.

The improved activity after cyclization of 8-substituted xanthines to thiazolpurines was noticed clearly in case of compounds AH-206 and AH-208 which were produced from cyclization of AH-201 and AH-203 respectively.

Introduction of ethylacetate moiety to compound AH-216 enhanced the activity of the produced AH-217 which could be a good lead for synthesis of new derivatives as antitumor agents.
AH-201, -206, R = H, AH-202, -207, R = Br, AH-203, -208, R = Cl,
AH-204, -209, R = Me AH-205, -210, R = NO₂

AH-211, R = NO₂, AH-212, R = Br, AH-213, R = Me, AH-214, R = Cl

4 (AH-216) 5 (AH-217)

Scheme 2: Structures of the tested compounds.
Table 1: Cytotoxic effects of the tested compounds against the human tumor cell lines SKW-3 and MDA-MB-231, as assessed by the MTT-dye reduction assay after 72 h continuous exposure.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} value (μM)</th>
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<tbody>
<tr>
<td></td>
<td>SKW-3^a</td>
</tr>
<tr>
<td>AH-201</td>
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<tr>
<td>AH-202</td>
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<tr>
<td>Cisplatin</td>
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</table>

^a Human T-cell leukemia; ^b Human breast cancer

REFERENCES


