

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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تم تصميم وتحضير مشتقات - الجديدة للبيورين- - ديون ومشتقات - للثيازولو [f -] بيورين- - ديون كمضادات محتملة للأورام السرطانية. تم اختبار المركبات الجديدة على نوعين من الخلايا السرطانية: يا سرطان الدم Skw-3 وخلايا سرطان الثدي MDA-231. قد ثبت أن المركب AH-206 أقوى المركبات تأثيراً وأظهر IC-50 تساوى ' , ميكرومول. لقد أظهرت الاختبارات أيضاً تأثيراً واضحاً للمركبات AH-201 AH-205 AH-208 AH-214 AH-217 كمضادات للسرطان.

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 IC_{50} values were calculated using commercially available software (GraphPad Prism). Compound AH-206 was the most potent cytotoxic agent among the newly synthesized compounds, with IC_{50} value of 17.3 μ M. 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 μ M.

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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 analogous to naturally occurring purine alkaloids. Many biological activities have been reported for these classes of compounds such as phosphodiesterase inhibitors¹, antimicrobial^{2&3}, anti-asthmatic^{4,6}, antiinflammatory agents⁷, antidiabetic through inhibition of phosphoenolpyruvate carboxykinase (PEPCK)⁸. Recently, we reported 1,8-disubstituted purine-2,6-diones as potent analgesic and anti-inflammatory agents through adenosine receptor antagonism^{9,11} and also 3,6-disubstituted thiazolo[2,3-*f*]purine-2,4-diones as potent analgesic and anti-inflammatory¹². 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*¹³⁻¹⁶. Moreover purine derivatives exhibited significant cytotoxic activity against several cancer cell lines^{17&18}. 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 II¹⁹. 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-*d*₆ was used as solvent and the chemical shifts are given in (ppm) values. The chemical shifts of the remaining protons of the deuterated solvent served as internal standard: 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)-1*H*-pyrimidine-2,4-dione **1**, 6-amino-3-(2-fluorobenzyl)-5-nitroso-1*H*-pyrimidine-2,4-dione **2** and 5,6-diamino-3-(2-fluorobenzyl)-1*H*-pyrimidine-2,4-dione **3** were prepared as we reported in a recent previous paper²⁰.

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) 5 (s, 2H, CH₂), 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), 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 C₁₂H₉FN₄O₂S: 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-2,3,6,7-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).

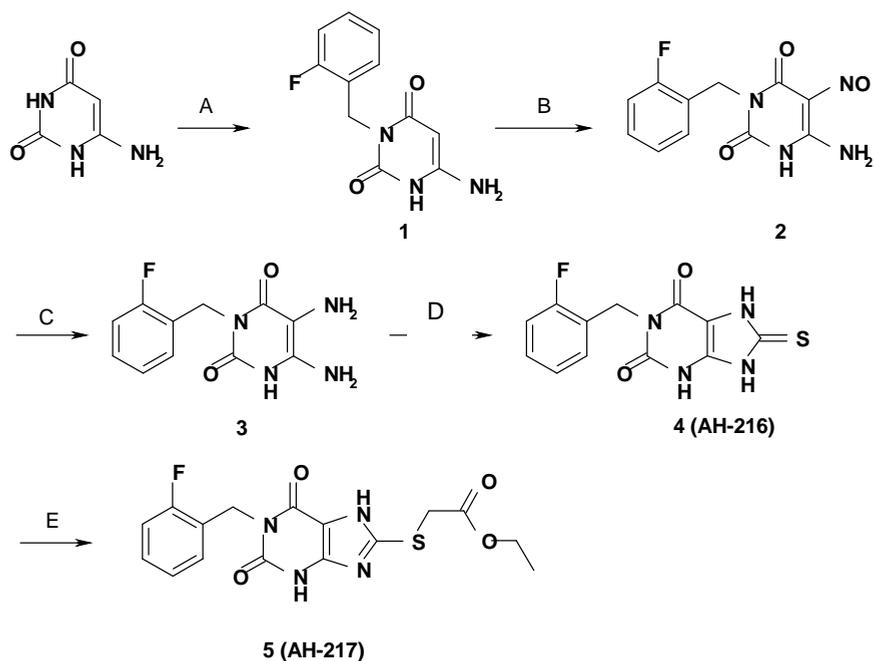
¹H-NMR, (500 MHz, DMSO-d₆) 1.2 (t, 3H, J= 7.3 Hz, CH₃), 4.1-4.2 (m, 4H, CH₂ & S-CH₂), 5 (s, 2H, CH₂), 7.0-7.2 (m, 3H, phenyl CH), 7.2-7.3 (m, 1H, phenyl CH), 12.0 (br s, 1H, N3-H), 13.5 (br s, 1H, N3-H).

¹³C-NMR: (125 MHz, DMSO-d₆), 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 C₁₆H₁₅FN₄O₄S⁺: 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) HMDMS, $(\text{NH}_4)_2\text{SO}_4$, 2-fluorobenzylbromide, $\text{Na}_2\text{S}_2\text{O}_3$, NaHCO_3 , reflux, (B) NaNO_2 , 50% CH_3COOH , heat 70°C , 30 min., (C) $\text{Na}_2\text{S}_2\text{O}_4$, 12.5% NH_3 , heat at $60\text{-}70^\circ\text{C}$, 20 min., (D) ethanol, KOH , CS_2 , 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 described²¹ with minor modifications²². Exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 µl/well) at a density of 1x10⁵ cells per ml and after 24h 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 micro-processor 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-1*H*-pyrimidine-2,4-dione **2** were prepared as we reported²⁰ (Scheme 1). 6-Amino-3-(2-fluorobenzyl)-1*H*-pyrimidine-2,4-dione **1**, was prepared by regioselective alkylation of 6-aminouracil in 1,1,1,3,3,3-hexamethylidisilazane (HMDS) as we reported²⁰. Then followed by nitrosation and reduction as described to obtain **(2)** and **(3)** respectively using reported procedure for similar derivatives²³.

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)-1*H*-pyrimidine-2,4-dione **3** with carbon disulfide in the presence of potassium hydroxide²⁴ 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 acetate in the presence of potassium hydroxide to give the required [1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-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-CH₂ at 4.2 and 61.7 ppm in ¹H-NMR and ¹³C-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 attacked²⁵.

Antitumor 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 IC₅₀ values were calculated using commercially available software (GraphPad Prism).

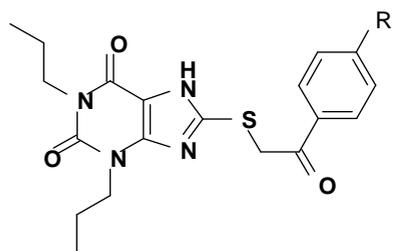
As evident from the results obtained (Table 1) the leukemic cell line was more responsive to the cytotoxic effects of the tested agents and actually all of the reached 50% inhibitory effects upon cellular survival and proliferation. Compound

AH-206 was the most potent cytotoxic agent among the newly synthesized compounds, with an 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. The other compounds were far less active against the T-cell leukemia SKW-3.

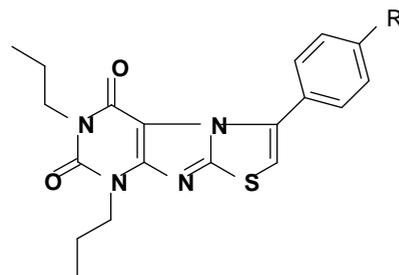
The other tumor model – the breast carcinoma-derived MDA-MB-231 cell line was less sensitive than SKW-3 and actually was found to be refractory to some of the compounds, which failed to induce 50% inhibition of cellular proliferation within the concentration range under evaluation (25-400 μM). Superior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

The improved activity after cyclization of 8-substituted xanthenes 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.

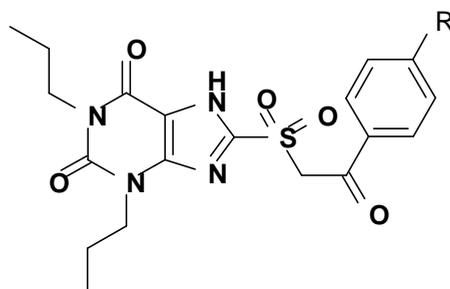


AH-201 to AH-205



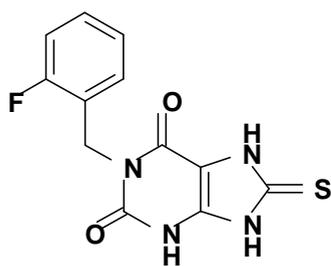
AH-206 to AH-210

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

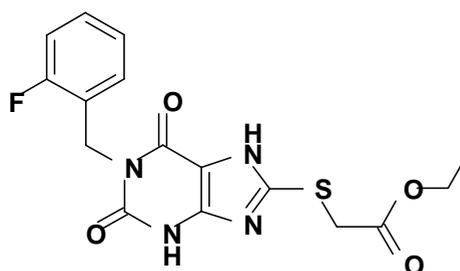


AH-211 to AH-214

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.

Compound	IC ₅₀ value (μM)	
	SKW-3 ^a	MDA-MB-231 ^b
AH-201	85.3 ± 2.2	329.3 ± 9.2
AH-202	146.7 ± 5.3	250.7 ± 7.7
AH-203	133.3 ± 2.7	366.7 ± 9.9
AH-204	148.0 ± 3.1	349.0 ± 8.2
AH-205	44.0 ± 2.2	372.1 ± 7.4
AH-206	17.3 ± 1.2	189.3 ± 5.9
AH-207	154.7 ± 6.2	> 400.0
AH-208	50.7 ± 2.7	116.2 ± 7.2
AH-209	141.3 ± 2.2	> 400.0
AH-210	309.3 ± 9.4	> 400.0
AH-211	168.0 ± 6.2	> 400.0
AH-212	277.3 ± 8.2	> 400.0
AH-213	302.7 ± 9.9	> 400.0
AH-214	76.1 ± 3.4	199.0 ± 5.2
AH-216	260.0 ± 8.9	> 400.0
AH-217	82.6 ± 1.9	237.0 ± 8.2
Cisplatin	6.7 ± 0.9	20.9 ± 3.1

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

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