Med Chem Res (2012) 21:1107–1119
DOI 10.1007/s00044-011-9612-6

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anticancer, anti-HIV-1, and antimicrobial activity of some tricyclic triazino and triazolo[4,3-e]purine derivatives

Fawzia A. Ashour · Samia M. Rida · Soad A. M. El-Hawash · Mona M. ElSemary · Mona H. Badr

Received: 5 May 2010 / Accepted: 21 February 2011 / Published online: 27 March 2011

Abstract In an effort to etablish new candidates with improved antineoplastic, anti-HIV-1 and antimicrobial activities, the synthesis of some new triazino and triazolo[4,3-e]purine derivatives is described: 6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H, 8H)-diones **3**–**6**; 5,7,9-trimethyl-1,2,4-triazolo[4,3-e]purine-6,8(5H, 7H, 9H)-diones **11**–**13**, together with the synthesis of the 8-substituted purine derivative: 8-(3,5-diamino-1H-pyrazol-4-yl)diazenyl-1,3-dimethyl-1H-purine-2,6(3H, 7H)-dione **7**. The prepared compounds were tested for their in vitro anticancer, anti-HIV and antimicrobial activities. The results of the in vitro anticancer screening revealed that compound **3** exhibited considerable activity against melanoma MALME-3 M, non-small lung cancer HOP-92 and breast cancer T-47D (GI_{50} values of 25.2, 31.8, and 32.9 μM, respectively). The anti-HIV-1 results indicated that compounds **7** and **13c** displayed moderate activity (maximum % cell protection 30.52 and 35.54 at 2×10^{-4} M, respectively). The in vitro antimicrobial data showed that compound **12** was the most active against *P. aeruginosa*, it was equipotent to ampicillin (MIC < 100 μg/ml). While compound **11d** was the most active against *P. vulgaris*, it was as active as ampicillin (MIC < 50 μg/ml). In addition, compounds **12** and **13c** were the most active against *S. aureus* (MIC <50 and <25 μg/ml, respectively). On the other hand, the tested compounds devoid of antifungal activity except **6b** and **11c** which showed weak activity against *A. niger*.

Keywords Purines · Anticancer · Anti-HIV · Antimicrobial activity

Introduction

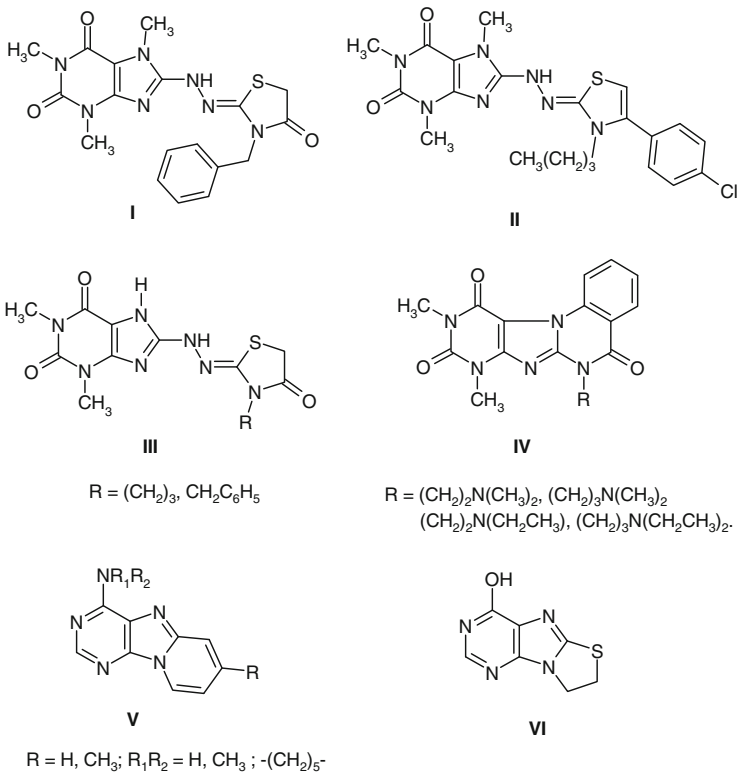
Purines and condensed purines have received much attention over the years for their interesting pharmacological properties as antineoplastic (Peifer *et al.*, 2009; Ito *et al.*, 2007; Lech-Maranda *et al.*, 2006), antileukemic (Ramasamy *et al.*, 1990; Avery *et al.*, 1990; Woo *et al.*, 1992; Steurer *et al.*, 2006; Jeha and Kantarjian, 2007), anti-HIV-1 (McLaren *et al.*, 1991; Johnson *et al.*, 1991; Valiaeva *et al.*, 2006), antiviral (Lee *et al.*, 1999; Li *et al.*, 2005; Kmonickova *et al.*, 2006; ElAshry *et al.*, 2006; Chen *et al.*, 2007) and animicrobial (Zinchenko *et al.*, 1987; Kascatan-Nebioglu *et al.*, 2006) agents.

This study is a continuation to previous efforts (Rida *et al.*, 2005, 2007) aiming to locate novel synthetic lead compounds for future development as anticancer, antiviral and/or antimicrobial agents. In our earlier study (Rida *et al.*, 2007), we reported the synthesis and evaluation of in vitro anticancer, anti-HIV-1 and antimicrobial activities of a number of new 8-substituted methylxanthines. The compounds were designed to comprise the purine nucleus linked at C-8 with various heterocyclic ring systems either directly or through a two-nitrogen atom spacer. Among these derivatives, 8-[(3-benzyl-4-oxo-thiazolidin-2-ylidene) hydrazino]-1,3,7-trimethyl-3,7-dihydropurine-2,6-dione (**I**, Fig. 1) exhibited a supersensitivity profile toward leukemia K-562 with a GI_{50} value < 0.01 μM, 8-{[3-butyl-4-(4-chlorophenyl)-2,3-dihydrothiazol-2-ylidene]hydrazino}-1,3,

Electronic supplementary material The online version of this article (doi:10.1007/s00044-011-9612-6) contains supplementary material, which is available to authorized users.

F. A. Ashour (✉) · S. M. Rida · S. A. M. El-Hawash · M. M. ElSemary · M. H. Badr
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, AR, Egypt
e-mail: Fawziaashour@yahoo.com

Fig. 1 Lead purine structures



7-trimethyl-3,7-dihydro-purine-2,6-dione (**II**, Fig. 1) displayed a moderate anti-HIV-1 activity, and 8-[(3-substituted-4-oxo-thiazolidin-2-ylidene)hydrazino]-1,3-dimethyl-3,7-dihydropurine-2,6-diones (**III**, Fig. 1) were 2–4 times more potent than ampicillin against *P. aeruginosa*.

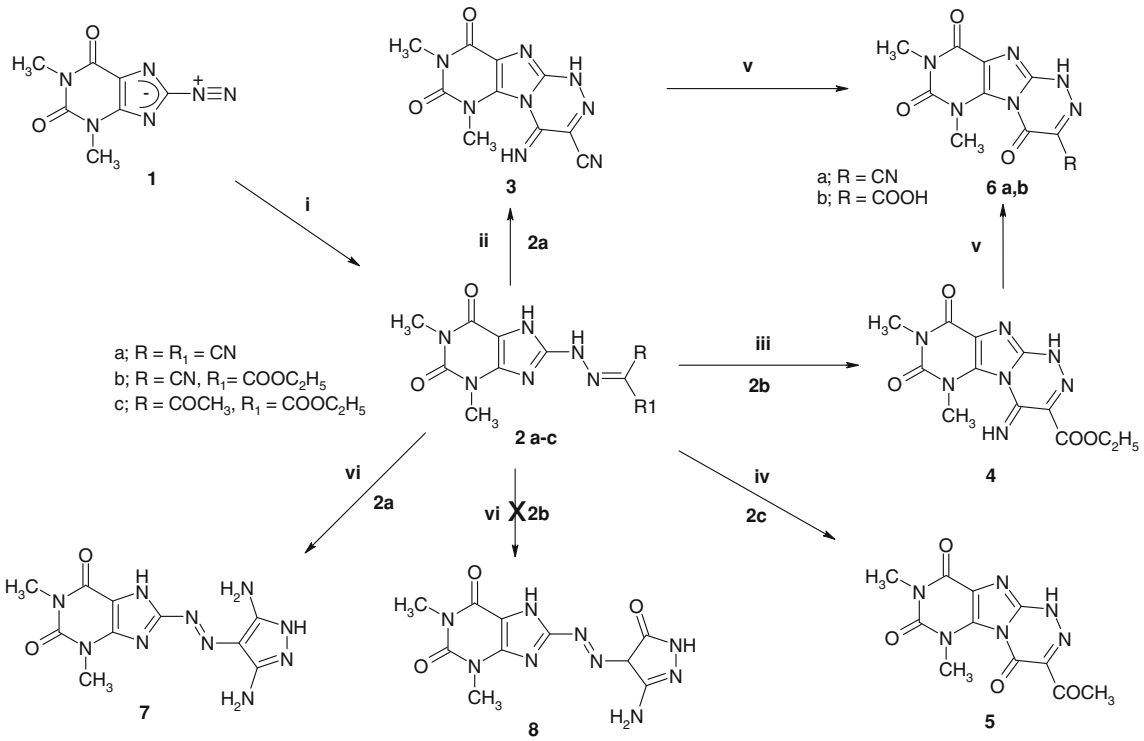
Moreover, methylxanthines, including caffeine, pentoxifylline and theophylline are compounds used worldwide. Many known biological effects of methylxanthines were reported in the literature. They have been found to enhance the cytocidal and growth-inhibitory effects of DNA-damaging agents such as anticancer activity of some chemotherapeutic agents, UV light and ionizing irradiation (Saito *et al.*, 2003; Lazarczyk *et al.*, 2004). On the other hand, methylxanthines have been recently shown to protect cells against the cytostatic or cytotoxic effects of several aromatic compounds and significantly decrease the mutagenicity of the anticancer aromatic drugs such as daunomycin, dixorubicin and mitoxantrone (Piosik *et al.*, 2005). Furthermore, some reports indicated that methylxanthines changed the inhibitory effect of antibacterial agents (Charles and Rawal, 1973; Banerjee and Chatterjee, 1981; Hosseinzadeh *et al.*, 2006). Aminophylline and caffeine potentiated the antimicrobial action of *penicillin G*, carbenicillin, ceftizoxime and gentamicin against *Staphylococus aureus* and *Pseudomenous aeruginosa* (Charles and Rawal, 1973; Hosseinzadeh *et al.*, 2006). Also, caffeine increased the efficacy of furazolidone against *Vibrios* (Banerjee and Chatterjee, 1981).

In addition, some polycyclic fused purine derivatives have been reported as potent anticancer or antiviral agents. For example, 6-dialkylaminoalkyl-8,10-dimethylpurino [7,8-a]quinazoline-5,9,11(6H, 8H, 10H)triones (**IV**, Fig. 1) exhibited significant in vitro cytotoxic activity against human promyelocytic leukemia and cervix adenocarcinoma (Settimo *et al.*, 1998). 4-Substituted pyrido[1,2-e]purine derivatives (**V**, Fig. 1) showed interesting activity on multidrug resistant cell lines, MCF7R, which were shown to have increased resistance to doxorubicin (Pinguet *et al.*, 1999). 7,8-Dihydrothiazolo[2,3-b]purin-4-ol (**VI**, Fig. 1) showed in vitro inhibiting effect on influenza virus(Hadden *et al.*, 1986). 1,3,8,10-Tetramethylpurino [7,8-g]-6-azapteridine-2,4,7,9(1H,3H,8H,10H)-tetrone was found to be active against P 338 Leukemia (Ueda *et al.*, 1987). 4-Amino-tetrahydroquinazolino [3,2-e] purine derivatives showed antiproliferative effects on the murine leukemia L1210 cell line (Verones *et al.*, 2010). Oligo and polyribonucleotides containing selected triazolo [2,3-a] purines were moderately active against HIV but showed greater potency against human cyclomelagovirus (HCMV) than ganciclovir (Tutonda *et al.*, 1998). These findings, together with the fact that the majority of DNA intercalating agents comprising a planar tricyclic or tetracyclic chromophore(Palmer *et al.*, 1988; Filippatos *et al.*, 1994; Kimura *et al.*, 1992; Abadi *et al.*, 1999), motivated our interest toward the design and synthesis of some triazino and triazolo[4,3-e]purine derivatives to explore their

anticancer, antiviral and antimicrobial activities hoping to go a step forward in the field of antimetabolities. Two new series of substituted 6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H, 8H)-diones **3**; **4**; **5** and **6a**, **b** (Scheme 1) and 5,7,9-trimethyl-1,2,4-triazolo[4,3-e] purine-6,8(5H, 7H, 9H)-diones **11a–d**; **12** and **13a–c** (Scheme 2). These compounds are considered as related structural analogs of the previously reported **IV**, **V** and **VI** (Fig. 1). In addition, 8-(3,5-diamino-1H-pyrazol-4-yl)diazenyl-1,3-dimethyl-1H-purine-2,6(3H, 7H)-dione **7** (Scheme 1) was designed as another molecular variant of **I**, **II**, and **III** (Fig. 1). The prepared compounds were biologically evaluated for their anticancer, anti-HIV-1 and antimicrobial activities to explore the effect of such molecular modifications on the anticipated pharmacological effects.

Chemistry

The target compounds were prepared following the synthetic pathways depicted in Schemes 1 and 2. The key intermediates hydrazono derivatives **2a–c** were prepared in good yields, as previously reported (Jones and Robins, 1960), by coupling an alcoholic suspension of 8-diazotheophylline **1** with the active methylene of malononitrile, ethyl cyanoacetate or ethyl acetoacetate in dry pyridine. The 8-diazo-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride **1** was prepared following the previously reported procedure (Jones and Robins, 1960), by diazotization of 8-amino-1,3-dimethyl-3,7-dihydropurine-2,6-dione hydrochloride with sodium nitrite in 5% hydrochloric acid at 0–5°C. Refluxing ethanolic solution of the hydrazono derivative **2a** afforded the respective 4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9-(6H,8H)-dione-3-carbonitrile **3**. However, ethyl (4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9 (6H,8H)-dione)-3-carboxylate **4** was obtained by refluxing **2b** in dimethyl formamide instead of ethanol. 3-Acetyl-4-oxo-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H,8H)-dione **5** was prepared in an excellent yield by refluxing a solution of the hydrazono derivative **2c** in absolute ethanol in the presence of equivalent amount anhydrous sodium acetate as a catalyst. However, cyclization failed in absence of sodium acetate, even on using boiling dimethyl formamide as a solvent. Hydrolysis of the imino derivative **3** or **4** in 18% hydrochloric acid afforded the corresponding oxo derivative **6a** or **b**, respectively. 8-(3,5-diamino-1H-pyrazol-4-yl)diazenyl-1,3-dimethyl-1H-purine-2,6(3H, 7H)-dione **7** was obtained in a good yield by treating **2a** with hydrazine hydrate in dry dimethyl formamide at room temperature. Attempts to prepare compound **8**, by cyclocondensation of **4b** with

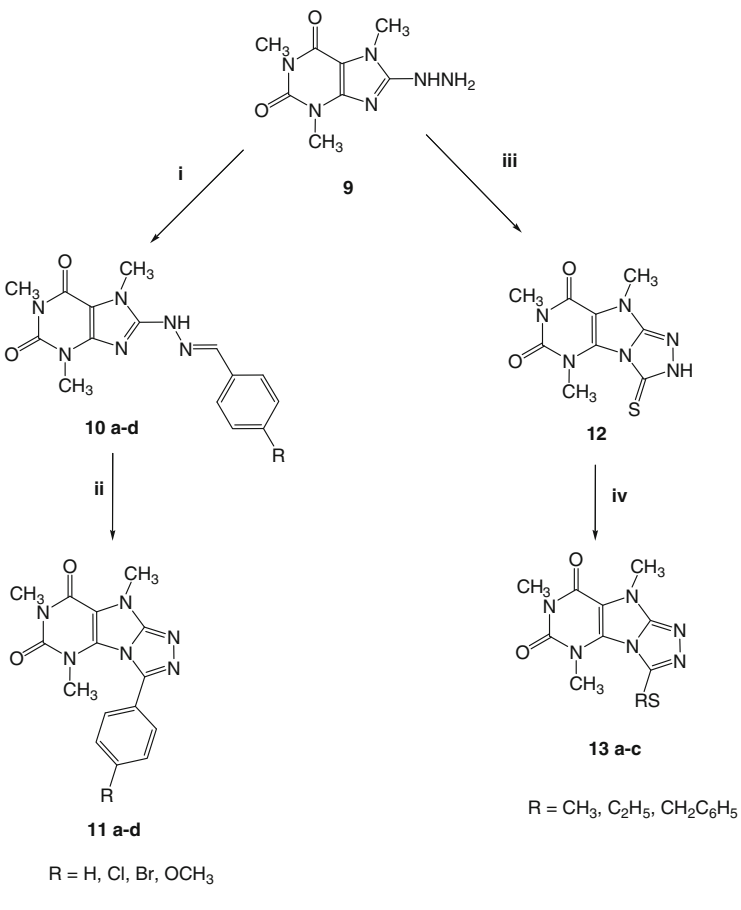


Scheme 1 Synthetic route for the synthesis of compounds **2a–c**, **3**, **4**, **5**, **6a**, **b** and **7**

hydrazine hydrate, following the reaction condition described for compound **7**, failed and the result was the recovery of the starting material. However, raising the reaction temperature from ambient to reflux gave the unexpected compound **4** (Confirmed by mixed m.p. with compound **4**, IR and ^{1}H-NMR spectra).

Scheme 2 starts with 8-hydrazinocaffeine **9** which was prepared in a good yield, as previously reported (Priewe and Poljak,1955), by refluxing the corresponding 8-chloro derivative with hydrazine hydrate in ethanol. Condensation of **9** with the appropriate aromatic aldehydes in boiling ethanol yielded the corresponding 8-arylidenehydrazino-3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-diones **10a-d**, as previously described (Klosa, 1956). Oxidative cyclization of **10a–d** using bromine in the presence of equivalent amount of anhydrous sodium carbonate afforded the corresponding 3-aryl-5,7,9-trimethyl-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-diones **11a–d**. On the other hand, 5,7,9-trimethyl-3-thioxo-2,3-dihydro-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-dione **12** was obtained by refluxing ethanolic solution of **9** with carbon disulfide in the presence of equivalent amount of sodium hydroxide. Alkylation of **12** with different alkyl halides in dimethyl formamide in the presence of equivalent amount of anhydrous potassium carbonate gave the respective 3-alkylthio or aralkylthio-5,7,9-trimethyl-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-diones **13a–c**.

The structures of the synthesized compounds were confirmed by microanalyses, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data (experimental section). ^{1}H-NMR spectrum of compound **2c** showed two singlets at 7.19 and 12.88 ppm due to two NH protons, indicating the existence of this compound in the hydrazono form rather than the azo form. ^{1}H-NMR spectrum of compounds **3** and **4** showed two deuterium oxide-exchangeable singlets at different chemical shifts attributed to two NH protons, confirming that these compounds exist in the imino form rather than the amino form. IR and ^{1}H-NMR spectra of compounds **6a**, **b** revealed the existence of three possible tautomeric forms. The IR spectrum showed OH and NH stretching absorption bands and the ^{1}H-NMR showed two NH and one OH signals at different chemical shifts each is integrated for 1/3 proton. IR spectrum of compound **12** revealed a broad band at 3442 cm^{-1} due to NH stretching and its ^{1}H-NMR



Scheme 2 Synthetic route for the synthesis of compounds **10a–d**, **11a–d**, **12** and **13a–c**

showed a deuterium oxide exchangeable singlet at 6.73 ppm attributed to NH proton, confirming the existence of this compound in the thione rather than the thiol form.

Experimental

All melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus (Sanyo) and were uncorrected. The IR spectra were recorded using KBr discs on a Perkin-Elmer 1430 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ^{1}H-NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer (Varian Inc., Palo Alto, CA, USA) or JNM-LA 400 FT NMR system (JEOL, Tokyo, Japan) using tetramethylsilane (TMS) as internal standard and dimethyl sulfoxide (DMSO-d_6) as solvent. Splitting patterns were assigned as follows: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet (chemical shift δ ppm). The ^{13}C-NMR spectra were performed on Joel spectrometer (500 MH_Z) using tetramethylsilane (TMS) as internal standard and dimethylsulfoxide (DMSO-d_6) as a solvent. MS were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV, Thermo Electron Corporation). The microanalyses were performed at the Microanalytical Laboratory, National Research Center, Cairo, and the data were within 0.4% of the theoretical values. Reactions were monitored by thin layer chromatography on silica gel-protected aluminium sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV-lamp at γ 254 nm for few seconds.

Synthesis of 3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-ylhydrazono malononitrile **2a**, ethyl (3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-ylhydrazono)cyanoacetate **2b**, and ethyl 2-[(3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-yl)hydrazono]-3-oxobutanoate **2c**

To an ice-cooled suspension of 8-diazotheophylline **1** (1.03 g, 5 mmole) in absolute ethanol (25 ml), a solution of malononitrile, ethyl cyanoacetate or ethyl acetoacetate (7.5 mmole) in dry pyridine (25 ml) was added dropwise while stirring over a period of half an hour. The reaction mixture was then stirred at room temperature for 3 h. The separated product was filtered, washed with water then ethanol and air dried (Table 1).

Table 1 Physical and analytical data of the synthesized compounds (**2–13**)

Comp. No.	R	R_1	Yield (%)	MP (Crys. Solv.)	Mol. formula[a] (mol. wt.)
2a	CN	CN	70	165–167	$C_{10}H_8N_8O_2$ (272.22)
2b	CN	$COOC_2H_5$	97	209–211[b]	–
2c	$COCH_3$	$COOC_2H_5$	41	161–163	$C_{13}H_{16}N_6O_5$ (336.31)
3	–	–	78	>350 (EtOH)	$C_{10}H_8N_8O_2$ (272.22)
4	–	–	72	234–236 (EtOH)	$C_{12}H_{13}N_7O_4$ (319.28)
5	–	–	94	>350 (DMF/EtOH)	$C_{11}H_{10}N_6O_4$ (290.24)
6a	CN	–	96	272–274 (EtOH)	$C_{10}H_7N_7O_3$ (273.21)
6b	COOH	–	96	224–226 (EtOH)	$C_{10}H_8N_6O_5$.HCl (328.67)
7	–	–	67	>350 (DMF/EtOH)	$C_{10}H_{12}N_{10}O_2$ (304.27)
10a	H	–	82	273–275[c] (EtOH)	$C_{15}H_{16}N_6O_2$ (312.33)
10b	Cl	–	91	260–262 (DMF)	$C_{15}H_{15}ClN_6O_2$ (346.77)
10c	Br	–	89	265–267 (DMF)	$C_{15}H_{15}BrN_6O_2$ (391.22)
10d	OCH_3	–	74	262–264[c] (EtOH)	$C_{16}H_{18}N_6O_3$ (342.35)
11a	H	–	68	240–242 (EtOH)	$C_{15}H_{14}N_6O_2$ (310.31)
11b	Cl	–	58	237–239 (EtOH)	$C_{15}H_{13}ClN_6O_2$ (344.76)
11c	Br	–	52	248–250 (EtOH)	$C_{15}H_{13}BrN_6O_2$ (389.21)
11d	OCH_3	–	85	225–227 (Dioxane)	$C_{16}H_{16}N_6O_3$ (340.34)
12	–	–	45	281–283 (EtOH/Ether)	$C_9H_{10}N_6O_2S$ (266.28)
13a	CH_3	–	85	188–190 (EtOH)	$C_{10}H_{12}N_6O_2S$ (280.31)
13b	CH_2CH_3	–	77	164–166 (EtOH)	$C_{11}H_{14}N_6O_2S$ (294.33)
13c	$CH_2C_6H_5$	–	63	132–134 (EtOH)	$C_{16}H_{16}N_6O_2S$ (356.40)

[a] Analyzed for C, H, N and the results are within ±0.4% of the theoretical values

[b] Jones and Robins, 1960

[c] Klosa, 1956

IR of compound **2a** (υ cm^{-1}): 3225 (br.NH); 2206 (C≡N); 1711 (C=O purine); 1666, 1552, 1503 (C=N, NH bending, C=C).

IR of compound **2b** (υ cm^{-1}): 3258, 3164 (NH); 2219 (C≡N); 1711 (br.C=O ester and C=O purine); 1656, 1614, 1540 (C=N, NH bending, C=C); 1236, 1140, 1053 (C–O–C).

IR of compound **2c** (υ cm^{-1}): 3276, 3201(NH); 1726 (C=O ester); 1697 (C=O ketone and C=O purine); 1649, 1563, 1525 (C=N, NH bending, C=C); 1239, 1139, 1067 (C–O–C).

^{1}H-NMR of compound **2c** (DMSO-d$_6$, δ ppm, Varian Gemini 200 MHz): 1.29 (t, $J = 7$ Hz, 3H, CH_2–$C\underline{H}_3$); 2.22 (s, 3H, $COC\underline{H}_3$); 3.23 (s, 3H, purine-N_3–$C\underline{H}_3$); 3.41 (s, 3H, purine-N_1–$C\underline{H}_3$); 4.24 (q, $J = 7$ Hz, 2H, $C\underline{H}_2$–CH_3); 7.19, 12.88 (two s, each 1H, two N$\underline{H}$, D_2O exchangeable).

Synthesis of 4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9-(6H,8H)-dione-3-carbonitrile **3**

A solution of 3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-ylazomalononitrile **2a** (0.54 g, 2 mmole) in absolute ethanol (10 ml) was heated under reflux for 15 min then left to cool at room temperature. The separated crystalline product was filtered, dried and recrystallized from ethanol (Table 1).

IR (υ cm^{-1}): 3310 (br.NH); 2242 (C≡N); 1697 (C=O purine); 1637, 1502, 1471 (C=N, NH bending, C=C).

^{1}H-NMR (DMSO-d$_6$, δ ppm, JNM-LA 400 FT): 3.30 (s, 3H, purine-N_3-$C\underline{H}_3$); 3.54 (s, 3H, purine-N_1–$C\underline{H}_3$); 9.99, 10.45 (two s, each 1H, two N$\underline{H}$, D_2O exchangeable).

Synthesis of ethyl (4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H,8H)-dione)-3-carboxylate **4**

A solution of ethyl (3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-yl azo)cyanoacetate **2b** (0.64 g, 2 mmole) in dry dimethyl-formamide (10 ml) was heated under reflux for 2 h then left to cool to room temperature. The precipitate formed after addition of few drops of water was filtered, dried and crystallized from ethanol (Table 1).

IR (υ cm^{-1}): 3306, 3174 (NH); 1718, 1700 (C=O ester and C=O purine respectively); 1637, 1592, 1514, 1468 (C=N, NH bending, C=C); 1237, 1171, 1068 (C–O–C).

^{1}H-NMR (DMSO-d$_6$, δ ppm, JNM-LA 400 FT): 1.36 (t, $J = 7$ Hz, 3H, CH_2–$C\underline{H}_3$); 3.30 (s, 3H, purine-N_3–$C\underline{H}_3$); 3.54 (s, 3H, purine-N_1–$C\underline{H}_3$); 4.41 (q, $J = 7$ Hz, 2H, $C\underline{H}_2$–CH_3); 9.25, 10.71 (two s, each 1H, two N$\underline{H}$, D_2O exchangeable). ^{13}C-NMR (DMSO-d6, δ ppm):13.77 (O = C–CH_2–$\underline{C}H_3$ ester); 61.13(O–$\underline{C}H_2$–CH_3 ester);162.89 ($\underline{C}$=O ester), 153.97($\underline{C}_3$-COOEt triazinopurine); 139.47 ($\underline{C}_4$=NH iminotriazinopurine); 135.96(N-$\underline{C}_{5a}$=C_{9a} triazinopurine) 32.26, 28.98(N_3–$\underline{C}H_3$, N_1–$\underline{C}H_3$ purine, respectively); 151.38, 154.89($\underline{C}_2$=O, $\underline{C}_4$=O purine, respectively); 114.58 (N–$\underline{C}_{9a}$=C_{5a} triazinopurine); 145.24(N_{10}–$\underline{C}_{10a}$=N_1–H triazinopurine).

Electron impact Mass Spectrum m/z (% abundance): 320 (2) M +1; 319 (9) M; 275 (6); 247 (30); 195 (100); 178 (5); 163 (9); 152 (11); 138 (16); 120 (9); 110 (10); 109 (8); 108 (7), 106 (7); 94 (9); 93 (12); 83 (5); 82 (40); 81 (12); 80 (13); 78 (9); 69 (5); 68 (28); 67 (49); 66 (6); 58 (13); 57 (5); 56 (10); 55 (8); 54 (14); 53 (29).

Synthesis of 3-acetyl-4-oxo-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H,8H)-dione **5**

To a solution of ethyl 2-[(3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-yl)azo]-3-oxobutanoate **2c** (0.67 g, 2 mmole) in absolute ethanol (10 ml), anhydrous sodium acetate (0.16 g, 2 mmole) was added. The reaction mixture was heated under reflux for 1 h and left to cool to room temperature. The separated crystals were filtered, washed with water, dried and recrystallized from dimethylformamide/ethanol (Table 1).

IR (υ cm^{-1}): 3344, 3257 (NH); 1732 (C=O triazinone); 1695, 1690 (C=O purine and C=O ketone, respectively); 1655, 1590, 1476 (C=N, NH bending, C=C).

^{1}H-NMR (DMSO-d$_6$, δ ppm, JNM-LA 400 FT): 2.44 (s, 3H, $COC\underline{H}_3$); 3.16 (s, 3H, purine-N_3-$C\underline{H}_3$); 3.42 (s, 3H, purine-N_1-$C\underline{H}_3$); 6.95 (s, 1H, N$\underline{H}$, D_2O exchangeable). ^{13}C-NMR (DMSO-d6, δppm):23.14(O=C–$\underline{C}H_3$ acetyl); 194.57 ($\underline{C}$=Oacetyl); 154.17($\underline{C}_3$–$COCH_3$ triazinopurine); 193.23 ($\underline{C}_4$=O oxotriazinopurine); 136.15(N–$\underline{C}_{5a}$=C_{9a} triazinopurine) 32.32,29.12 (N_3-$\underline{C}H_3$, N_1-$\underline{C}H_3$ purine, respectively); 151.41, 154.92($\underline{C}_2$=O, $\underline{C}_4$=O purine, respectively); 114.62 (N-$\underline{C}_{9a}$=C_{5a} triazinopurine); 145.12(N_{10}–$\underline{C}_{10a}$=N_1–H triazinopurine).

Synthesis of 4-oxo-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H,8H)-dione-3-carbonitrile **6a**, and 4-oxo-6,8-dimethyl-1,4-dihydro-1,2,4-triazino[4,3-e]purine-7,9(6H,8H)-dione-3-carboxylic acid **6b**

A solution of 4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino-[4,3-e]purine-7,9(6H,8H)-dione-3-carbonitrile **3** or ethyl (4-imino-6,8-dimethyl-1,4-dihydro-1,2,4-triazino [4,3-e]purine-7,9(6H,8H)-dione)-3-carboxylate **4** (2 mmole) in 18% hydrochloric acid (10 ml) was heated under reflux for 1 h. The reaction mixture was concentrated under reduced pressure and left to cool to room temperature. The separated crystalline product was filtered, dried and recrystallized from ethanol (Table 1).

IR of compound **6a** (ν cm^{-1}): 3440, 3166, 3133 (br.OH, NH); 2243 (C≡N); 1727 (C=O triazinone); 1694 (C=O purine); 1664, 1597, 1500, 1459 (C=N, NH bending, C=C).

^{1}H-NMR of compound **6a** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.24 (s, 3H, purine-N_3-C$\underline{H}_3$); 3.46 (s, 3H, purine-N_1-C$\underline{H}_3$); 6.93, 7.06, 7.19 (three s, each $^1/_3$ H, O$\underline{H}$, N$\underline{H}$, N$\underline{H}$-N, D_2O exchangeable).

Electron impact mass spectrum of compound **6a** m/z (% abundance): 274 (8) M + 1; 273 (58) M; 244 (8); 217 (6); 216 (20); 215 (8); 189 (16); 188 (22); 161 (17); 152 (13); 136 (15); 109 (9); 108 (21); 94 (8); 93 (7); 82 (23); 81 (17); 80 (10); 79 (11); 78 (9); 69 (13); 68 (56); 67 (100); 66 (12); 58 (11); 56 (31); 55 (24); 54 (24); 53 (38); 52 (9).

IR of compound **6b** (υ cm^{-1}): 3522–3441, 3126 (br.OH, br.NH); 1726 (C=O triazinone); 1699 (br.C=O acid and C=O purine); 1664, 1646, 1569, 1535 (C=N, NH bending, C=C).

^{1}H-NMR of compound **6b** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.19 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.38 (s, 3H, purine-N_1–C$\underline{H}_3$); 6.98, 7.11, 7.20 (three s, each $^1/_3$ H, O$\underline{H}$, N$\underline{H}$, N$\underline{H}$–N, D_2O exchangeable); 13.19 (s, 1H, COO$\underline{H}$, D_2O exchangeable).

Synthesis of 8-(3,5-diamino-1H-pyrazol-4-yl)diazenyl-1,3-dimethyl-1H-purine-2,6(3H, 7H)-dione **7**

To an ice-cooled suspension of 3,7-dihydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-yl azomalononitrile **2a** (0.54 g, 2 mmole) in dry dimethylformamide (5 ml), hydrazine hydrate (98%) (0.5 g, 10 mmole) was added dropwise with stirring. After complete addition, the reaction mixture was left overnight at room temperature, poured onto ice/water and neutralized with dilute hydrochloric acid. The separated product was filtered, washed with water, dried, and crystallized from dimethylformamide/water (Table 1).

IR (ν cm^{-1}): 3445, 3328, 3239 (NH_2, NH); 1677 (C=O purine); 1645, 1569, 1484 (C=N, NH bending, C=C).

^{1}H-NMR (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.26 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.47 (s, 3H, purine-N_1–C$\underline{H}_3$); 6.58 (br.s, 4H, two N$\underline{H}_2$, D_2O exchangeable); 11.22, 12.45 (two s, each 1H, N$\underline{H}$ purine, N$\underline{H}$ pyrazole, D_2O exchangeable).

Synthesis of 8-arylidenehydrazino-3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-diones **10a–d**

To a suspension of 8-hydrazinocaffeine **9** (1.12 g, 5 mmole) in absolute ethanol (20 ml), the appropriate aromatic aldehyde (5 mmole) was added. The reaction mixture was heated under reflux for 30 min then cooled to room temperature. The separated solid was filtered, washed with ethanol, dried and crystallized from the proper solvent (Table 1).

IR of compounds **10a–d** (υ cm^{-1}): 3177–3115 (NH); 1697–1691 (C=O); 1645–1624, 1623–1596, 1578–1574, 1548–1538 (C=N, NH bending, C=C).

^{1}H-NMR of compound **10a** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.19 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.37 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.92 (s, 3H, purine-N_7–C$\underline{H}_3$); 7.37–7.44 (m, 3H, Ar–$C_{3,4,5}$–$\underline{H}$); 7.65 (d, 2H, Ar–$C_{2,6}$–$\underline{H}$), 8.09 (s, 1H, N=C$\underline{H}$); 11.45 (s, 1H, N$\underline{H}$, D_2O exchangeable).

^{1}H-NMR of compound **10b** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.17 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.33 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.85 (s, 3H, purine-N_7–C$\underline{H}_3$); 7.45 (d, J = 8.4 Hz, 2H, Ar–$C_{2,6}$–$\underline{H}$); 7.65 (d, J = 8.4 Hz, 2H, Ar–$C_{3,5}$–$\underline{H}$); 8.07 (s, 1H, N=C$\underline{H}$); 11.50 (s, 1H, N$\underline{H}$, D_2O exchangeable).

Synthesis of 3-aryl-5,7,9-trimethyl-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-diones **11a–d**

To a stirred mixture of 8-arylidenehydrazino-3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-diones **10a–d** (2 mmole) and anhydrous sodium carbonate (2.3 g, 2 mmole) in chloroform (20 ml), bromine (0.3 ml) was added. The reaction mixture was stirred at room temperature for 2 h, evaporated under reduced pressure. The residue was triturated with ice-cold water, filtered, washed with water, dried and crystallized from the proper solvent (Table 1).

IR of compounds **11a–d** (υ cm^{-1}): 1713–1707 (C=O); 1676–1664, 1645–1637, 1533–1525, 1482–1469 (C=N, C=C).

^{1}H-NMR of compound **11a** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 2.88 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.31 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.83 (s, 3H, purine-N_7–C$\underline{H}_3$); 7.53–7.60 (m, 3H, Ar–$C_{3,4,5}$–$\underline{H}$); 7.68 (d, 2H, Ar–$C_{2,6}$–$\underline{H}$).

^{1}H-NMR of compound **11b** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 2.95 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.37 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.83 (s, 3H, purine-N_7–C$\underline{H}_3$); 7.62 (d, J = 8 Hz, 2H, $C_{2,6}$–Ar–$\underline{H}$); 7.72 (d, J = 8 Hz, 2H, $C_{3,5}$–Ar–$\underline{H}$).

^{1}H-NMR of compound **11c** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 2.96 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.25 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.83 (s, 3H, purine-N_7–C$\underline{H}_3$); 7.65 (d, J = 8 Hz, 2H, $C_{2,6}$–Ar–$\underline{H}$); 7.76 (d, J = 8 Hz, 2H, $C_{3,5}$–Ar–$\underline{H}$).

Synthesis of 5,7,9-trimethyl-3-thioxo-2,3-dihydro-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-dione **12**

To a mixture of 8-hydrazinocaffeine **9a** (2.24 g, 10 mmole) and sodium hydroxide (0.4 g, 10 mmole) in absolute ethanol (20 ml), carbon disulphide was added (30 ml). The reaction mixture was heated under reflux for 24 h and then

the solvent was evaporated under reduced pressure. The residue was dissolved in water, filtered and the filterate was neutralized with concentrated hydrochloric acid. The formed precipitate was filtered, washed with water, dried and crystallized from ethanol/ether (Table 1).

IR (υ cm^{-1}): 3442 (br.NH); 2631 (weak SH); 1715 (C=O); 1684, 1489, 1467 (C=N, C=C); 1549, 1331, 1035, 970 (N–C=S amide I, II, III, IV bands).

^{1}H-NMR (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.16 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.33 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.60 (s, 3H, purine-N_7–C$\underline{H}_3$); 6.73 (s, 1H, N$\underline{H}$, D_2O exchangeable).

Synthesis of 3-alkylthio or aralkylthio- 5,7,9-trimethyl-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-diones **13a–c**

A. mixture of 5,7,9-trimethyl-3-thioxo-2,3-dihydro-1,2,4-triazolo[4,3-e]purine-6,8(5H,7H,9H)-dione **12** (0.53 g, 2 mmole), anhydrous potassium carbonate (0.28 g, 2 mmole) and the appropriate alkyl halide (3 mmole) in dry dimethyl formamide (5 ml) was stirred at room temperature for 3 h. The reaction mixture was poured onto ice/water and the formed precipitate was filtered, washed with water, dried and crystallized from ethanol (Table 1).

IR of compounds **13a–c** (υ cm^{-1}): 1711–1698 (C=O); 1664–1652, 1550–1535, 1452–1449 (C=N, C=C); 1220–1215, 1036–1035 (C–S–C).

^{1}H-NMR of compound **13a** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 2.68 (s, 3H, S–C$\underline{H}_3$); 3.19 (s, 3H, purine-N_3–C$\underline{H}_3$); 3.40 (s, 3H, purine-N_1–C$\underline{H}_3$); 3.73 (s, 3H, purine-N_7–C$\underline{H}_3$).

Biological activity

Anticancer screening

Materials and methods

Anticancer screening was performed at the National Cancer Institute (NCI), Bethesda, Maryland, USA. The compounds were evaluated in three cell lines in a one-dose primary anticancer assay subsequent to the NCI preclinical antitumor drug discovery screen (Grever *et al.*, 1992; Boyed and Paull, 1995). The three cell lines used were lung (NCI-H460), breast (MCF-7) and CNS (SF-268). In the current protocol, each cell is inoculated and preincubated on microtiter plates. Test agents are then added at a single concentration (100 μM), and the culture is incubated for 48 h. Endpoint determinations are made with alamar blue. The results for each agent are presented as the percent of growth of the treated cells compared to the untreated control cells. Compounds which reduced the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for the evaluation in the full panel in vitro antitumor screen consisting of 60 human tumor cell lines, derived from nine clinically isolated types of cancer types (Leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer) following the NCI preclinical antitumor drug discovery screen. Each compound was tested at five concentrations at ten-fold dilutions. A 48 h continuous drug exposure protocol was used and a sulforodamine B (SRB) protein assay was used to estimate cell viability or growth (Boyed and Paull, 1995).

Results

Four of the synthesized compounds (**3**; **4**; **11a**, **b**) were selected by the National Cancer Institute (NCI) and evaluated for their in vitro antineoplastic activity against three-cell-line panel consisting of the Breast-MCF-7 cell line, the lung-NCI-H460 cell line and the CNS-SF-268 cell line. Only compound **3** showed promising activity. It reduced the growth of breast cell line to less than 32% (14%) (Table 2). Compound **3** was then subjected to the NCI in vitro disease-oriented human cells screening panel assay (Grever *et al.*, 1992; Boyed and Paull, 1995) to investigate its antitumor activity. About 60 cell lines of nine tumor subpanels were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration versus % growth inhibition curves. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value corresponds to the compound's concentration causing 50% decreases in net cell growth. The TGI value is the compound's concentration resulting in total growth inhibition and the LC_{50} is the compound's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI or LC_{50} values of all cell lines in subpanel and fullpanel, respectively.

Compound **3** exhibited considerable activity against some of the tested cell lines (Table 3). For example, GI50 values of 31.8 μM against non-small cell lung cancer HOP-92, 25.2 μM against melanoma MALME-3 M and 32.9 μM against breast T-47D.

The GI50, TGI, and LC50 subpanel and full panel mean-graph midpoint (MG-MID) values, respectively, are shown (Table 4). The ratio obtained by dividing the compound's full panel MG-MID (μM) by its individual subpanel MG-MID (μM) has been considered as a measure of compound

Table 2 Growth percentages of the 3-cell line panel in primary anticancer screen of some selected compounds

Comp. no.	NSC no.	Sample concentration	Growth percentages		
			Lung NCI-H460	Breast MCF7	CNS SF-268
3	S-720606	1.00E−04 Molar	62	14	47
4	S-720605	1.00E−04 Molar	99	93	98
11a	S-720609	1.00E−04 Molar	98	84	90
11b	S-720610	1.00E−04 Molar	95	82	90

Table 3 Growth inhibitory action (GI_{50}) of some selected in vitro tumor cell lines (μM)

Comp. no.	NCS no.	Panel	Subpanel cell lines (cytotoxicity GI_{50} in μM)[a]
3	S-720606	Melanoma	MALME-3M (25.2), SK-MEL-5 (45.9)
		Lung cancer	HOP-92 (31.8), NCI-H226 (54.0)
		Breast cancer	T-47D (32.9)
		Renal cancer	ACHN (44.3), A498 (51.4), CAKI-1 (51.7), UO-31 (50.6)
		Leukemia	CRF-CEM (67.8)

[a] Data obtained from NCI in vitro disease-oriented human cell screen

Table 4 Median growth inhibitory concentrations (GI_{50}, μM), Median total growth inhibitory concentrations (TGI, μM) of in vitro subpanel tumor cell lines, and selectivity ratios of compound **3** toward the nine tumor cell lines

Subpanel tumor cell lines[a]	GI_{50} (μM)	TGI (μM)	Selectivity ratios
I	94.6	100	0.90
II	87.3	100	0.97
III	100	100	0.85
IV	98.0	100	0.87
V	81.6	96.9	1.04
VI	97.9	100	0.87
VII	72.2	100	1.18
VIII	86.3	100	0.99
IX	91.6	100	0.93
Full panel MG-MID	85.1[b]	100[c] (100)[d]	–

[a] I, Leukemia; II,non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer

[b] GI_{50} (μM) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent

[c] TGI (μM) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent

[d] LC_{50} (μM) full panel mean-graph mid-point (MG-MID)

selectivity (Monks *et al.*, 1991). Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective (Monks *et al.*, 1991). Accordingly, compound 3 was non-selective with ratios ranging between 0.85 and 1.18 (Table 4).

In vitro anti-HIV-1 activity

Materials and methods

The in vitro anti-HIV drug testing system was performed in the National Cancer Institute's Developmental Therapeutics Program, AIDS antiviral screening program, according to a reported procedure (Weislow *et al.*, 1989). The assay involved the killing of T_4 lymphocytes by HIV. T_4 lymphocytes (CEM cell line) were exposed to HIV at a virus-to-cell ratio of approximately 0.05 and treated with the compounds, dissolved in dimethylformamide, at doses ranging from 10^{-8} to 10^{-4} M. A complete cycle of virus reproduction is necessary to obtain the required cell killing (incubation at 37°C in a 5% carbon dioxide atmosphere for 6 days). Uninfected cells with the compound served as a toxicity control, whereas the infected and uninfected cells without the compound served as basic controls. After incubation, the tetrazolium salt XTT was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Formazan production was measured spectrophotometrically and possible protective activity was confirmed by microscopic detection of viable cells. The effect of each compound on cell growth of HIV-infected and uninfected cells was compared to that of untreated uninfected cells. All tests were compared with AZT as positive control carried out at the same time under identical conditions.

Table 5 Maximum % Protection, the corresponding dose (molar) and IC_{50} (molar) of the selected compounds

Comp. no.	NCS no.	Maximum % protection	Dose (M)	IC_{50} (M)
5	722234-U/1	1.43	2.00×10^{-5}	$>2.00 \times 10^{-4}$
6a	722233-T/1	10.90	2.00×10^{-4}	$>2.00 \times 10^{-4}$
6b	722232-S/1	3.73	2.00×10^{-5}	$>2.00 \times 10^{-4}$
7	722235-V/1	30.52	2.00×10^{-4}	$>2.00 \times 10^{-4}$
13a	722239-Z/1	4.86	2.00×10^{-4}	$>2.00 \times 10^{-4}$
13c	722240-A/1	35.54	2.00×10^{-4}	$>2.00 \times 10^{-4}$

Results

Six compounds (**5**; **6a**, **b**; **7**; **13a**, **c**) have been selected by NCI and evaluated for their effects on HIV-1 induced cytopathogenicity in a human T_4 lymphocyte cell line (CEM) (Weislow *et al.*, 1989). Activity is expressed as % of protection which represents the percentage of surviving HIV-infected cells treated with the test compound (at the indicated concentration) relative to the same uninfected untreated controls. The effective concentration 50% (EC_{50}), represents the concentration of the test agent resulting in 50% reduction of viral cytopathic effect. The 50% inhibitory concentration (IC_{50}), represent the toxic concentration of drug resulting in 50% growth inhibition of normal uninfected cells. In this screen, the compounds are considered to be active if they display complete protection at a concentration <0.1 μM. Compounds which show incomplete protection or show protection at a concentration above 0.1 μM are considered moderately active. As revealed from (Table 5), compounds **7** and **13c** showed moderate reduction of viral cytopathic effect by 30.52 and 35.54% at 2.00×10^{-4} M, respectively. The other tested compounds were inactive.

Antimicrobial evaluation

Materials and methods

Inhibition zones measurement

The tested compounds were evaluated by the agar cup diffusion technique (Conte and Barriere, 1988), using a 2 mg/ml solution in DMF. The test organisms were *Staphylococcus aureus* (NCTC 4163) and *Bacillus subtilis* (ATTC 6633) as Gram-positive bacteria, *Pseudomonas aeruginosa* (ATTC 9027), *Escherichia coli* (5933), and *Salmonella typhi* (ATCC 13311) as Gram-negative bacteria and *Proteus vulgaris* (ATTC 49132) as spore forming Gram-negative bacteria. They were also evaluated for their in vitro antifungal activity against four types of fungi, *Candida albicans* (NCTC 2708) and *Saccharomyces cervisiae* (ATTC 9763) as examples of yeast; *Asperigillus niger* (ATTC 16404) and *Asperigillus terreus* (local isolate) as examples of true fungi. Each 100 ml of sterile molten agar (at 45°C) received 1 ml of 6 h broth and then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 ml of the 2 mg/ml solution of the tested compounds. The plates were then incubated at 37°C for 24 h for bacteria or 48 h for fungi. A control using DMF without the test compound was included for each organism. Ampicillin in DMF was used as standard antibacterial, while clotrimazol was used as antifungal reference.

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentration (MIC) of the most active compounds was measured using the two-fold serial broth dilution method (Scott, 1989). The test organisms were grown in their suitable broth for 24 h for bacteria and 48 h for fungi at 37°C. Twofold serial dilutions of the test compounds solution were prepared using the suitable broth to obtain concentrations 200, 100, 50, and 25 μg/ml. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculum and were incubated at 37°C for 48 h. Then the tubes were observed for the presence or absence of microbial growth.

Results

Compounds (**3**; **4**; **5**; **6a**, **b**; **7**; **11a–d**; **12**; **13a–c**) were preliminary evaluated for their in vitro antibacterial activity. The results recorded in (Table 6) revealed that the tested compounds exhibited promising activity toward the Gram-negative *P. aeruginosa* and *P. vulgaris*. Compound **12** was the most active against *P. aeruginosa*, it was equipotent to ampicillin (MIC < 100 μg/ml), while compounds **11b**, **11c**, **11d**, and **13a** showed half the activity. On the other hand, compound **11d** was the most active against *P. vulgaris*, it was as active as ampicillin (MIC < 50 μg/ml). Compounds **4**, **5**, **6a**, **11b**, and **11c** displayed half the

Table 6 The inhibition zones (IZ) in mm diameter and minimal inhibitory concentration (MIC) in μg/ml of the tested compounds against different bacterial strains

Comp. no.	*S. aureus*		*B. subtilis*		*S. typhi*		*P. aeruginosa*		*E. coli*		*P. vulgaris*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
3	–	–	14	–	12	–	18	–	14	–	19	–
4	16	–	14	–	–	–	20	–	15	–	22	<100
5	–	–	14	–	–	–	–	–	14	–	23	<100
6a	14	–	–	–	–	–	16	–	12	–	22	<100
6b	14	–	14	–	–	–	16	–	13	–	21	<200
7	–	–	10	–	–	–	–	–	–	–	14	–
11a	–	–	15	–	11	–	17	–	14	–	19	–
11b	22	<100	13	–	–	–	21	<200	12	–	25	<100
11c	14	–	11	–	–	–	20	<200	12	–	22	<100
11d	14	–	20	<200	17	–	21	<200	20	<200	28	<50
12	24	<50	11	–	–	–	21	<100	12	–	19	–
13a	20	<200	–	–	–	–	20	<200	12	–	19	–
13b	20	<200	13	–	–	–	19	–	12	–	19	–
13c	26	<25	–	–	–	–	19	–	12	–	18	–
Ampicillin	–	5	–	5	–	100	–	100	–	10	–	50

Table 7 The inhibition zones (IZ) in mm diameter and minimal inhibitory concentration (MIC) in μg/ml of the tested compounds against fungi

Comp. no.	*C. albicans*		*S. cerevisiae*		*A. niger*		*A. terreus*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
3	12	–	14	–	12	–	–	–
4	17	–	15	–	–	–	–	–
5	13	–	13	–	–	–	–	–
6a	15	–	14	–	–	–	–	–
6b	13	–	14	–	20	<200	–	–
7	11	–	12	–	–	–	–	–
11a	12	–	15	–	16	–	–	–
11b	12	–	14	–	–	–	–	–
11c	14	–	13	–	22	<100	–	–
11d	18	–	16	–	–	–	16	–
12	11	–	12	–	–	–	–	–
13a	11	–	12	–	–	–	–	–
13b	11	–	13	–	–	–	–	–
13c	11	–	14	–	–	–	–	–
Clotrimazole	–	5	–	5	–	10	–	10

potency of ampicillin, while compound **6b** showed weak activity (one fourth the activity). Furthermore, determination of the antibacterial activity against the Gram-positive *S. aureus* indicated that compounds **12** and **13c** exhibited significant activity but lower than that of ampicillin (MIC < 50 and <25 μg/ml, respectively).

Considering the antifungal activity, the tested compounds were devoid of activity except **6b** and **11c**, which showed weak activity against *A. niger* (Table 7).

Discussion

From the previously mentioned results, it could be deduced that compound **3** exhibited considerable activity against melanoma MALME-3M, non-small lung cancer HOP-92 and breast cancer T-47D. Moreover, significant antibacterial activity was associated with the 3-aryl-1,2,4-triazolo[4,3-e]purine series **11a–d**. Maximum activity was achieved when the substituent at position 3 was 4-methoxyphenyl group **11d**. Replacement of 3-aryl moiety in (compounds **11a–d**) by 3-alkylthio or aralkylthio (compounds **13a–c**) decreased the activity toward the Gram-negative *P. aeruginosa* and *P. vulgaris* and increased the activity toward the Gram-positive *S. aureus*. Maximum activity was obtained when the substituent at position 3 was benzylthio **13c**. Substituted 1,2,4-triazino[4,3-e]purines **4**, **5**, and **6a** showed promising activity against the spore forming Gram-negative bacteria *P. vulgaris*.

It is worthy to mention that compounds **11b**, **12**, and **13a** exhibited broad spectrum of activity against Gram-positive and Gram-negative bacteria.

Acknowledgments The authors with to thank Dr. Manal A. Shalaby, Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubark City for Scientific Research and Technology

Application, Borg El-Arab, Alexandria, Egypt for performing the antibacterial and antifungal screening. The authors are also grateful to the staff of the Department of Health and Human Services, National Cancer Institute, Bethesda, Maryland, USA for the anti-cancer and anti-HIV reports.

References

Abadi AH, El-Subbagh HI, Al-Khamees HA (1999) Synthesis, antitumor and antitubercular evaluation of certain new xanthenone and acridinone analogs. Arzneim-Forsch Drug Res 49(1): 259

Avery TL, Finch RA, Vasquez KM, Radparver S, Hanna NB, Revankar GR, Robins RK (1990) Chemotherapeutic characterization in mice of 2-amino-9-β-d-ribofuranosylpurine-6-sulfinamide (sulfinosine), a novel purine nucleoside with unique antitumor properties. Cancer Res 50:2625–2630

Banerjee SK, Chatterjee SN (1981) Radiomimetic property of furazolidone and the caffeine enhancement of its lethal action on the vibrios. Chem Biol Interact 37:321–335

Boyed MR, Paull KD (1995) Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Dev Res 34:91–109

Charles BG, Rawal BD (1973) Synergistic effect of methyl-substituted xanthines and neomycin sulphate on *Staphylococcus aureus and Pseudomonas aeruginosa* in vitro. Lancet 1:971–973

Chen ZY, Cheng AC, Wang MS, Xu DW, Zeng W, Li Z (2007) Antiviral effects of PNA in duck hepatitis B virus infection model. Acta Pharmacol Sin 28(10):1652–1658

Conte JE, Barriere SL (1988) Manual of antibiotics and infectious diseases, 1st edn. Lea and Febiger, USA

ElAshry ES, Rashed N, Abdel-Rahman A, Awad LF, Rashed HA (2006) Synthesis of 2-bromomethyl-3-hydroxy-2-hydroxymethylpropyl pyrimidine and theophylline nucleosides under microwave irradiation. Evaluation of their activity against hepatitis B virus. Nucleosides Nucleotides Nucl Acids 25(8): 925–939

Filippatos E, Papadaki-Valiraki A, Todoulou O, Jacquemin-Sablon A (1994) Synthesis of *N*-(9H-xanthen-9-yl)aminoalkanamide and *N*-(9H thioxanthen-9-yl)aminoalkanamide derivatives and their in vitro evaluation as potential intercalators and antitumor drugs. Arch Pharm 327:61–66

Grever MR, Schepartz SA, Chabner BA (1992) The National Cancer Institute: cancer drug discovery and development program. Semin Oncol 19:622–638

Hadden JW, Simon LN, Giner-Sorolla A (1986) Dihydrothiazolo derivatives. Pat. Specif. AU 554: 372; through Chem Abstr 108, 37511s (1988)

Hosseinzadeh H, Bazzaz BSF, Sadati MM (2006) In vitro evaluation of methylxanthines and some antibiotics: interaction against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Iran Biomed J 10(3):163–167

Ito S, Koshikawa N, Mochizuki S, Takenaga K (2007) 3-Methyladenine suppresses cell migration and invasion of HT1080 fibrosarcoma cells through inhibiting phosphoinositide 3-kinases independently of autophagy inhibition. Int J Oncol 31(2): 261–268

Jeha S, Kantarjian H (2007) Clofarabine for the treatment of acute lymphoblastic leukemia. Expert Rev Anticancer Ther 7(2):113–118

Johnson VA, Merril DP, Videler JA, Chou TC, Byington RE, Eron JJ, D'Aquila RT, Hirsch MS (1991) Two-drug combinations of zidovudine, didanosine, and recombinant interferon-α. A inhibit replication of zidovudine-resistant human immunodeficiency virus type 1 synergistically in vitro. J Infect Dis 164:646–655

Jones JW, Robins RK (1960) Potential purine antagonists. The preparation and reactions of some 8-diazopurines. J Am Chem Soc 82:3773–3779

Kascatan-Nebioglu A, Melaiye A, Hindi K, Durmus S, Panzner MJ, Hogue LA, Mallett RJ, Hovis CE, Coughenour M, Crosby SD, Milsted A, Ely DL, Tessier CA, Cannon CL, Youngs WJ (2006) Synthesis from caffeine of a mixed *N*-heterocyclic carbene-silver acetate complex active against resistant respiratory pathogens. J Med Chem 49(23):6811–6818

Kimura M, Kato A, Okabayashi I (1992) Acridine derivatives. IV. Synthesis, molecular structure, and antitumor activity of the novel 9-anilino-2,3-methylenedioxyacridines. J Heterocycl Chem 29:73–80

Klosa J (1956) Condensation of caffein hydrazide-8 with aldehydes and ketones. Arch Pharm 289(4): 211–217; through Chem Abstr 51: 7383f (1957)

Kmonickova E, Potmesil P, Holy A, Zidek Z (2006) Purine P1 receptor-dependent immunostimulatory effects of antiviral acyclic analogues of adenine and 2,6-diaminopurine. Eur J Pharmacol 530:179–187

Lazarczyk M, Grzela T, Niderla J, Lazarczyk MA, Milewski L, Dziunycz P, Skopinski P, Golab J (2004) Differential influence of pentoxifylline on murine colon adenocarcinoma and melanoma-derived metastatic tumor development in lungs. Oncol Rep 11(5):1121–1125

Lech-Maranda E, Korycka A, Robak T (2006) Pharmacological and clinical studies on purine nucleoside analogs-new anticancer agents. Mini Rev Med Chem 6:575–581

Lee K, Choi Y, Gullen E, Schuete-Wirtz S, Raymond F, Schinazi RF, Cheng Y, Chu CK (1999) Synthesis and anti-HIV and anti-HBV activities of 2′-fluoro-2′,3′-unsaturated L-nucleosides. J Med Chem 42(7):1320–1328

Li Y, Fu L, Yeo H, Zhu JL, Chou CK, Kou YH, Yeh SF, Gullen E, Austin D, Cheng YC (2005) Inhibition of hepatitis B virus gene expression and replication by helioxanthin and its derivative. Antivir Chem Chemother 16(3):193–201

McLaren C, Datema R, Knupp CA, Buroker RA (1991) Didanosine. Antivir Chem Chemother 2(6):321–328

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 83:757–766

Palmer BD, Rewcastle GW, Atwell GJ, Baguley BC, Denny WA (1988) Potential antitumor agents. 54. Chromophore requirements for in vivo antitumor activity among the general class of linear tricyclic carboxamides. J Med Chem 31(4):707–712

Peifer C, Buhler S, Hauser D, Kinkel K, Totzke F, Schachtele C, Laufer S (2009) Design, synthesis and characterization of N_9/N_7-substituted 6-aminopurines as VEGF-R and EGF-R inhibitors. Eur J Med Chem 44(4):1788–1793

Pinguet F, Mavel S, Galtier C, Gueiffier A (1999) Synthesis and cytotoxicity of novel pyrido[1,2e]purines on multidrug resistant human MCF7 cell 54. Pharmazie 45(12):876–881

Piosik J, Gwizdek-Wisniewska A, Ulanowska K, Ochocinski J, Czyz A, Wegrzyn G (2005) Methylxanthines (caffine, pentoxifylline and theophlline) decrease the mutagenic effect of daunomycin, doxorubicin and mitoxanthrone. Acta Biochima Pol 52(4): 923–926

Priewe H, Poljak A (1955) 8-Hydrazino-purine and their conversion into pyrazolone. Chem Ber 88:1932–1937; through Chem Abstr 50, 12067d (1956)

Ramasamy K, Imamura N, Hanna NB, Finch RA, Avery TL, Robins RK, Revankar GR (1990) Synthesis and antitumor evaluation in

mice of certain 7-deazapurine (pyrrolo[2,3-d]pyrimidine) and 3-deazapurine (imidazo[4,5-c]pyridine) nucleosides structurally related to sulfenosine, sulfinosine, and sulfonosine. J Med Chem 33(4):1220–1225

Rida SM, Ashour FA, El-Hawash AM, El-Semary MM, Badr MH, Shalaby MA (2005) Synthesis of some novel benzoxazole derivatives as anticancer, anti-HIV-1 and antimicrobial agents. Eur J Med Chem 40:949–959

Rida SM, Ashour FA, El-Hawash AM, El-Semary MM, Badr MH (2007) Synthesis of some novel substituted purine derivatives as potential anticancer, anti-HIV and antimicrobial agents. Arch Pharm Chem Life Sci 340:185–194

Saito Y, Gopalan B, Mhashilkar AM, Roth JA, Chada S, Zumstein L, Ramesh R (2003) Adenovirus-mediated PTEN treatment combined with caffeine produces a synergistic therapeutic effect in colorectal cancer cells free. Cancer Gene Ther 10(11):803–813

Scott AC (1989) Laboratory cotrol of antimicrobial therapy. In: Collee JG, Duguid JP, Faster AG, Marmion BP (eds) Mackie and Mc-Cartney practical medical microbiology, vol 2, 13th edn. Churchill Livingstone, Edinburgh, pp 161–181

Settimo AD, Settimo FD, Marini AM, Primofiore G, Salerno S, Viola G, Via LD, Magno SM (1998) Synthesis, DNA binding and in vitro antiproliferative activity of purinoquinazoline, pyridopyrimidopurine and pyridopyrimidobenzimidazole derivatives as potential antitumor agents. Eur J Med Chem 33:685–696

Steurer M, Pall G, Richards S, Schwarzer G, Bohlius J, Greil R (2006) Single-agent purine analogues for the treatment of chronic lymphocytic leukaemia: a systematic review and meta-analysis. Cancer Treat Rev 32:377–389

Tutonda MG, Buckheit RW Jr, Agrawal VK, Broom AD (1998) Antiviral Oligo-and polyribonucleotides containing selected triazolo[2,3-a]purines. J Med Chem 41(25):4958–4964

Ueda T, Adachi T, Sakakibara J, Asano M, Nakagami J (1987) Synthesis, antitumor activity and vascular relaxing effect of purino[7, 8]-6-azapteridines and [1, 2, 4]triazino-[3, 2-f]purines. Chem Pharm Bull (Tokyo) 35(10):4031–4038

Valiaeva N, Beadle JR, Aldern KA, Trahan J, Hostetler KY (2006) Synthesis and antiviral evaluation of alkoxyalkyl esters of acyclic purine and pyrimidine nucleoside phosphonates against HIV-1 in vitro. Antiviral Res 72(1):10–19

Verones V, Flouquet N, Farce A, Carato P, Leonce S, Pfeiffer B, Berthelot P, Lebegue N (2010) Synthesis, biological evaluation and docking studies of 4-amino-teyrahydroquinazolono[3,2-e]purine derivatives. Eur J Med Chem 45:5678–5684

Weislow OW, Kiser R, Fine D, Bader J, Shoemaker RH, Boyd MR (1989) New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral. J Natl Cancer Inst 81(8): 577–586

Woo PWK, Kostlan CR, Sircar JC, Dong MK, Gilbertsen RB (1992) Inhibitors of human purine nucleoside phosphorylase. Synthesis and biological activities of 8-amino-3-benzylhypoxanthine and related analogs. J Med Chem 35(8):1451–1457

Zinchenko G, Kremzer AA, Strokin YV, Krasovskii AN, Steblyuk PN (1987) Synthesis and biological properties of ylidene derivatives of 7-(3-chloro-2-buten-1-yl)-8-hydrazinotheophylline. Farm Zh (Kiev) 3:39–41; through Chem Abstr, 108, 150134j (1988)