Full Paper

Synthesis of Some Novel Substituted Purine Derivatives As Potential Anticancer, Anti-HIV-1 and Antimicrobial Agents*

Samia M. Rida, Fawzia A. Ashour, Soad A.M. El-Hawash, Mona M. El-Semary, and Mona H. Badr

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt

In search of novel purine antimetabolites, a series of 8-substituted methylxanthine derivatives was prepared in order to explore their *in vitro* anticancer, anti-HIV-1 and antimicrobial activities. The target compounds include: 8-[(3-substituted-4-oxo-thiazolidin-2-ylidene)hydrazino]-1,3-dimethyl (or 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones **5a – e**, 8-[(3,4-disubstituted 2,3-dihydrothiazol-2-ylidene)hydrazino]-1,3,7-trimethyl-3,7-dihydropurine-2,6-diones **6a – d** and 8-(5-amino-3-arylpyrazol-1-yl)-1,3-dimethyl- (or 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones **7a – g**. The *in vitro* anticancer results revealed that compound **5d** exhibited a super sensitivity profile towards leukemia K-562 with a GI_{50} value of <0.01 μM. Compound **7c** showed significant activity against colon cancer HCT-15 and renal cancer CAKI-1 (GI_{50} values of 0.47 and 0.78 μM, respectively). Compound **7a** displayed high activity against colon cancer HCT-15 (GI_{50} = 0.8 μM). The anti-HIV-1 results indicated that compound **6b** displayed a good reduction of viral cytopathic effect (56.69%). The antimicrobial results showed that compound **5a** was four times more active than ampicillin against *P. aerugenosa* (MIC = <25 μg/mL), compound **5b** had twice the activity of ampicillin, while compounds **5d**, **7c** and **7f** were equipotent to ampicillin. On the other hand, compound **7a** was equipotent to ampicillin against *P. vulgaris* (MIC = 50 μg/mL).

Keywords: Anticancer / Anti-HIV / Antimicrobial activity / Methylxanthines / Purines

Received: July 29 2006; accepted: December 19, 2006

DOI 10.1002/ardp.200600118

Introduction

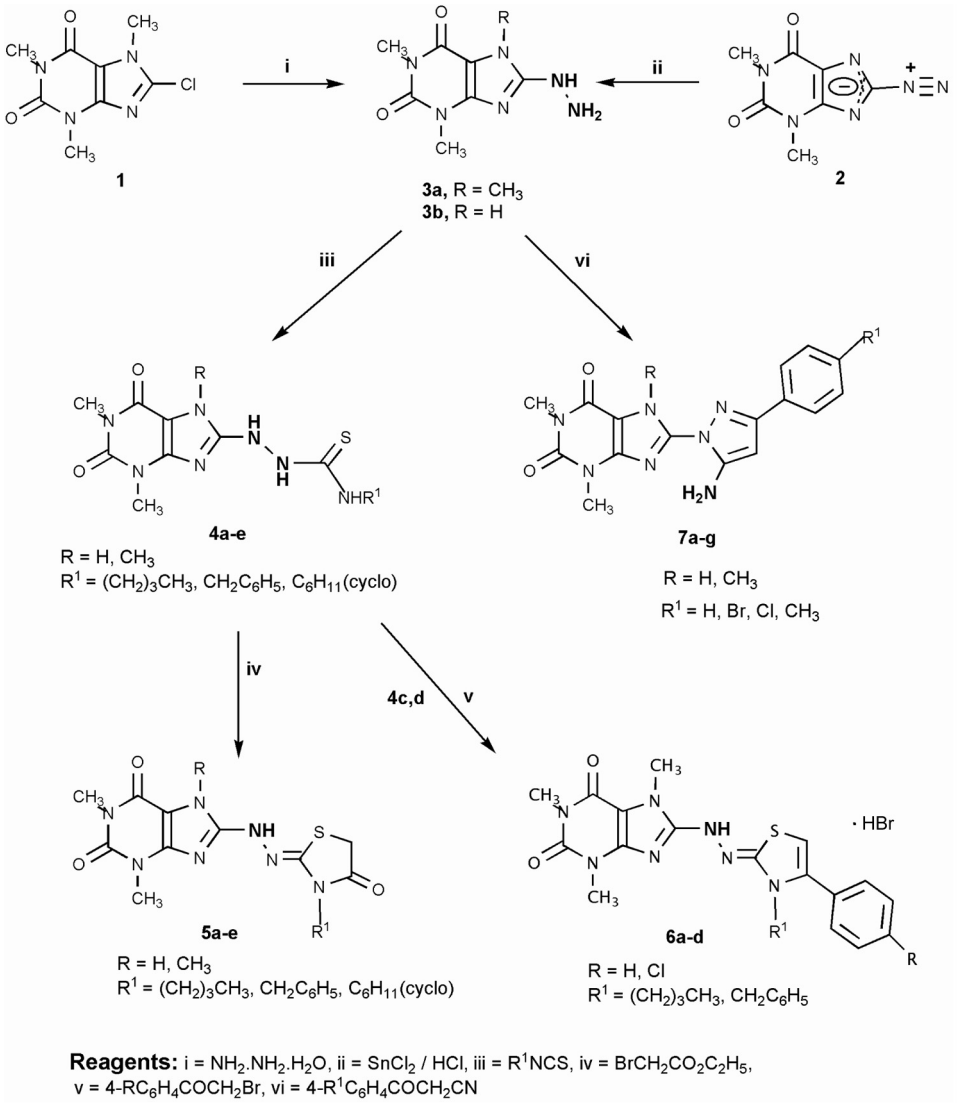
The purine ring system is considered to be one of the most important heterocyclic ring system in nature as it has the distinction of being the parent ring in countless derivatives of biological relevance. Therefore, it is not surprising that modified purines possess diverse biological activities, and lead to a better understanding of some biological effects of DNA-damaging agents as well as enzymes-substrate interactions. This lead has been used in the development of many potent medicinal agents. For example, the antineoplastic [1 – 4], antileukemic [5 – 9], anti-HIV-1 [10 – 12], antiviral [13 – 17], antibacterial and antifungal [3, 18] purine derivatives.

Moreover, methylxanthines (caffeine, pentoxifylline and theophylline) are well known to enhance the cytocidal and growth inhibitory effects of DNA-damaging agents such as radiation, UV light, and anticancer agents on tumor cells [19, 20]. On the other hand, it has been reported that methylxanthines may 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, doxorubicin, and mitoxantrone [20].

In view of the above mentioned findings and in the search for novel antimetabolite purine derivatives, our work has been focused on design and synthesis of a novel series of 8-substituted methylxanthine derivatives in order to evaluate their potential as anticancer, anti-HIV, and/or antimicrobial agents. The target compounds were

Correspondence: Soad A. M. EL-Hawash, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria AR Egypt
E-mail: soadhawash@yahoo.com
Fax: +20 3 487 3273

* Parts of this work have been presented at Assiut University 4th Pharmaceutical Sciences Conference, Assiut, Egypt, March 6-7, 2004; Poster P 97-98.



Scheme 1. Synthetic route for compounds **3–7.**

designed to incorporate a five-membered heterocyclic ring of biological interest either directly linked to the purine nucleus at position 8 or through a two-nitrogen atom spacer. The prepared compounds were biologically evaluated to test the effect of such molecular modification of purines on the anticipated pharmacological effects.

Results and discussion

Chemistry

The target compounds were prepared following the synthetic pathway depicted in Scheme 1. The intermediate 8-hydrazino-1,3,7-trimethyl-3,7-dihydropurine-2,6-dione **3a** was prepared in a good yield, as previously reported [21], by refluxing the corresponding 8-chloro derivative **1** with hydrazine hydrate in ethanol. On the other hand, reaction of 8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione with hydrazine hydrate did not afford the respective 8-hydrazino derivative **3b**, instead, a hydrazine salt of 8-chloro1,3-dimethyl-3,7-dihydropurine-2,6-dione was produced because of the acidic nature of hydrogen at position 7 as previously described [21].

Here, we report a new synthetic procedure to prepare **3b** in a good yield by reduction of the respective 8-diazo derivative **2** with stannous chloride in hydrochloric acid. The 8-diazo-1,3-dimethyl-3,7-dihydropurine-2,6-dione **2** was prepared following the previously reported procedure [22] 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 **3a** or **3b** with the selected isothiocyanate afforded the respective 8-(*N*-substituted-thiocarbamoylhydrazino)-1,3-dimethyl (or 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones **4a – e**. Reaction of **4a – e** with ethyl bromoacetate in a refluxing mixture of absolute ethanol and dry dioxan yielded the corresponding 8-[(3-substituted-4-oxo-thiazolidin-2-ylidene)hydrazino]-3,7-dihydropurine-2,6-diones **5a – e** as previously reported for the preperation of related compounds [23]. In addition, refluxing a mixture of **4c** or **4d** with the appropriate phenacylbromide gave the respective 8-[(3,4-disubstituted-2.3-dihydrothiazol-2-ylidene)hydrazino]-3,7-dihydropurine-2,6-diones **6a-d** as previously discussed for the preperation of analogous compounds [24, 25]. On the other hand, 8-(5-amino-3-arylpyrazol-1-yl)-3,7-dihydropurine-2,6-dione derivatives **7a – g** were obtained in good yield by refluxing **3a** or **3b** and the appropriate ω-cyanoacetophenone in ethanol containing acetic acid as previously reported for the preperation of related compounds [26]. The structures of the synthesized compounds were confirmed by microanalyses, IR, ^{1}H-NMR, and mass spectral data (Experimental section).

Biology

Anticancer activity

Antitumor activity was performed at the National Cancer Institute (NCI), Bethesda, Maryland, USA. Nine of the synthesized compounds **5a – d** and **7a, c, d, e, g** were selected by the NCI and subjected to the NCI *in vitro* disease-oriented human cells screening panel assay [27, 28]. 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}, TG1, 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 TG1 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.

In this study, the preliminary screening data of NCI indicated that seven compounds **5a, b, d** and **7a, c, e, g** showed antitumor activity (Tables 1 – 4). Compound **5d** exhibited super sensitivity profile towards leukemia K-562 with GI_{50} value resides in the nanomolar range (< 0.01 μM). Compound **7c** exhibited moderate to strong activity against all of the tested cell lines with GI_{50} of 10^{-6} to 10^{-5} μM. It showed significant activity against colon cancer HCT-15 and renal cancer CAKI-1 (GI_{50} values, 0.47 and 0.78 μM, respectively). Compound **7a** showed activity against most of the tested cell lines. It exhibited significant activity against colon cancer HCT-15 (GI_{50} = 0.8 μM). Compound **7e** displayed moderate activity against some of the tested cell lines, especially CNS cancer SF-268, melanoma MALME-3M, melanoma SK-MEL-5 and renal cancer 786-0 (GI_{50} values 9.91, 7.13, 6.36, and 6.90 μM, respectively). In addition, compound **5a** showed considerable activity towards some of the tested cell lines. For example, lung cancer HOP-62, NCI H322M, colon cancer KM12, ovarian cancer SK-OV-3, and CNS cancer SF-268, SF-295 (GI_{50} values, 4.63, 5.09, 6.36, 6.47, 7.47, and 7.35 μM, respectively). On the other hand, compounds **5b** and **7g** showed weak activity with GI_{50} of 10^{-5} to 10^{-4} μM. Tables 2 and 3 indicate the GI_{50}, TGI, LC_{50} subpanel and full panel mean-graph midpoint (MG-MID) values for the active compounds respectively.

The ratio obtained by dividing the compound full panel MG-MID (μM) by its individual subpanel MG-MID (μM) is considered as a measure for selectivity [29]. Ratios greater than 6 indicate selectivity toward the corresponding subpanel cell line, while ratios between 3 and 6 refer to moderate selectivity. Accordingly, the tested compounds proved to be non-selective except compound **5d** which had moderate selectivity for leukemia with ratio of 2.64 (Table 4).

From the above mentioned results it could be concluded that the antitumor activity was associated with 8-[(3-substituted-4-oxo-thiazolidin-2-ylidene)hydrazino]-1,3-dimethyl (or 1,3,7-trimethyl)-3,7-dihydropurine -2,6-diones **5a, b, d** and 8-(5-amino-3-arylpyrazol-1-yl)-1,3-dimethyl (and 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones **7a – g**. Regarding the 8-(5-amino-3-arylpyrazol-1-yl) series **7a – g**, compound **7a** showed significant activity with a broad spectrum of activities. Substitution at position 4 of the phenyl moity with methyl group resulted in an increase in activity **7c**, while substitution with chlorine atom decreased the activity **7e**. On the other hand, substitution at N-7 of purine nucleus with methyl group greatly decreased or destroyed the activity **7e** and **7g**. By comparing the 8-(3-substituted-4-oxo-thiazolidinyl) derivatives **5a, b, d**, maximum activity was observed when the substituent at position 3 of the thiazolidine ring was a butyl group **5a**. Also, substitution at N-7 of the purine nucleus with a methyl group greatly decreased the activity **5d**.

From these findings, we could concluded that maintaining a hydrogen at position 7 of purine nucleus might

Table 1. Growth inhibitory action (GI_{50}) of some selected *in vitro* tumor cell lines (μM).

N°	NCS	Panel	Subpanel cell lines (cytotoxicity GI_{50} in μM)[a)]
5a	**720616**	Leukemia	CCRF-CEM (22.20), MOLT-4 (21.80, SR (11.90)
		Lung Cancer	HOP-62 (6.63), NCI-H322M (5.09), NCI-H460 (13.70)
		Colon Cancer	HCT-116 (15.20), HT29 (23.90), KM12 (6.36)
		CNS Cancer	SF-268 (7.74), SF-295 (7.35), U 251 (15.00)
		Melanoma	SK-MEL-28 (20.00), M14 (28.5)
		Ovarian Cancer	OVCAR-4 (19.50), OVCAR-5 (14.50), SK-OV-3 (6.47)
		Renal Cancer	786-0 (17.30)
		Prostate Cancer	DU-145 (19.20
		Breast Cancer	MCF7 (13.70), MDA-MB-435 (14.70), MDA-N (14.6)
5b	**720617**	Leukemia	CCRF-CEM (22.10), SR (24.90)
		Lung Cancer	HOP-92 (15.70)
		CNS Cancer	SF-295 (21.20), SF-539 (29.10)
		Renal Cancer	786-0 (27.10)
		Breast Cancer	MCF7(19.40), HS 578T(17.10), MDA-MB-435 (19.10), MDA-N (16.40)
5d	**720619**	Leukemia	K-562 (< 0.01), RPMI-8226 (17.50)
		Lung Cancer	HOP-92 (5.91)
		CNS Cancer	SF-539 (25.60)
		Ovarian Cancer	OVCAR-3 (21.00), OVCAR-4 (26.30)
7a	**720611**	Leukemia	CCRF-CEM (2.64), K-562 (6.45), MOLT-4 (7.15), SR (6.31)
		Lung Cancer	A549/ATCC (5.78), EKVX (5.42), HOP-92 (6.46), NCI-H226 (5.54), NCI-322M (4.93), NCI-H460 (3.30)
		Colon Cancer	HCC-2998 (8.67), HCT-116 (6.49), COLO 205 (7.05), HCT-15 (0.80), HT29 (4.15), KM12 (6.58)
		CNS Cancer	SF-295 (4.92), SF-539 (5.08), U251(6.77)
		Melanoma	SK-MEL-5 (5.39), UACC-62 (6.37)
		Ovarian Cancer	IGROV1(1.34), OVCAR-3 (5.35), OVCAR-4 (2.91), OVCAR-8 (4.86), SK-OV-3 (4.03)
		Renal Cancer	786-0 (6.18), ACHN (3.66), CAKI-1(2.12), RXF 393 (2.68), SN12C (4.60), UO-31(6.89)
		Prostate Caner	DU-145 (7.92)
		Breast Cancer	NCI/ADR-RES (2.40), MDA-MB-231/ATCC (10.70), MDA-N (11.7), T-47D (11.30)
7c	**720612**	Leukemia	CCRF-CEM (2.65), HL-60(TB) (5.04), K-562 (4.03), MOLT-4 (2.06), RPMI-B226 (6.80), SR (2.43)
		Lung Cancer	A549/ATCC (4.68), EKVX (3.09), HOP-62 (3.28), NCI-H226 (6.71), NCI-322M (4.04), NCI-H460 (1.71)
		Colon Cancer	HCC-2998 (7.68), HCT-116 (2.68), COLO 205 (3.62), HCT-15 (0.47), HT29 (4.53), KM12 (4.88)
		CNS Cancer	SF-268 (3.59), SF-295 (2.69), SF-539 (4.23), SNB-19 (8.56), U251(5.36)
		Melanoma	MALME-3M (3.86), M14 (4.32), SK-MEl-2 (6.64), SK-MEl-28 (6.13),SK-MEL-5 (3.35), UACC-62(5.37)
		Ovarian Cancer	IGROV1(2.20), OVCAR-3 (4.55), OVCAR-4 (4.01), OVCAR-5 (8.05), OVCAR-8 (3.37), SK-OV-3 (3.31)
		Renal Cancer	786-0 (2.08), A498 (4.87), ACHN (1.92), CAKI-1(0.78), SN12C (5.32), TK-10 (9.84), UO-31(2.78)
		Prostate Cancer	DU-145 (6.35)
		Breast Cancer	MCF7(6.78), NCI/ADR-RES (1.55), MDA-MB-231/ATCC (8.97), MDA-N (5.99), T-47D (4.77), BT-549 (6.95), MDA-MB-435 (7.72)
7g	**720615**	Melanoma	SK-MEl-28 (23.10),SK-MEL-5 (32.40)
7e	**720614**	Leukemia	MOLT-4 (11.60), S (13.00)
		Lung Cancer	HOP-62 (10.50)
		Colon Cancer	HCT-116 (19.10)
		CNS Cancer	SF-268 (9.910), SF-295 (25.50), SF-539 (17.30)
		Melanoma	MALME-3M (7.13), SK-MEL-5 (6.36)
		Ovarian Cancer	OVCAR-4(5.60), OVCAR-8 (18.10), SK-OV-3 (18.10)
		Renal Cancer	786-0 (6.90)
		Breast Cancer	HS 578T(22.40)

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

Table 2. Median growth inhibitory concentrations (GI_{50}, μM) of *in vitro* subpanel tumor cell lines and GI_{50} (μM) full panel mean-graph mid-points (MG-MID).

N°	Subpanel tumor cell lines[a]									Full panel GI_{50} MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
5a	38.1	58.7	100	21.1	48.8	40.9	81.4	59.6	43.9	35.5
5b	36.4	74.7	73.8	47.8	67.8	60.9	90.9	82.8	45.6	54.9
5d	21.5	77.7	79.8	88.2	79.6	54.2	100	89.1	79.8	57.5
7a	7.53	6.28	5.62	7.02	10.3	6.63	9.22	13.8	11.8	6.76
7c	3.84	8.25	3.98	4.89	6.42	4.25	5.65	11.3	8.20	4.68
7e	60.3	74.4	86.5	50.5	68.4	53.6	88.4	100	67.9	53.7
7g	100	80.4	57.6	69.3	71.6	80.6	92.8	100	94.9	81.3

[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} full panel mean-graph midpoint (μM).

Table 3. Median total growth inhibitory concentrations (TGI, μM) of *in vitro* subpanel tumor cell lines, TGI (μM) full panel mean-graph mid-points (MG-MID) and LC_{50} (μM) full panel mean-graph mid-points (MG-MID).

N°	Subpanel tumor cell lines[a]									Full panel TGI MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
5a	76.3	92.1	100	90.6	100	88.5	92.7	100	84.7	85.1 (97.7)[c]
5b	90.9	98.6	100	100	100	100	98.6	100	79.1	93.3 (100)
5d	81.1	97.6	100	100	100	98.4	100	100	100	97.7 (100)
7a	92.2	73.3	98.1	81.8	48.4	74.8	72.5	72.5	82.9	69.2 (97.7)
7c	100	40.2	49.2	55.1	41.1	44.8	33.3	60.2	61.5	38.0 (85.1)
7e	100	98.0	100	93.9	97.6	98.5	100	100	100	97.7 (100)
7g	100	96.0	100	100	100	100	100	100	100	100 (100)

[a] For subpanel tumor cell lines see footnote ({) of Table 2.
[b] TG1 (μM) full panel mean-graph mid-point (MG-MID) = the average sensitivity of all cell lines towards the test agent.
[c] LC_{50} (μM) full panel mean-graph mid-point (MG-MID).

Table 4. Selectivity ratios of the active compounds toward the nine tumor cell lines.

N°	Subpanel tumor cell lines[a]								
	I	II	III	IV	V	VI	VII	VIII	IX
5a	0.93	0.60	0.36	1.68	0.73	0.87	0.44	0.60	0.81
5b	1.51	0.73	0.74	1.15	0.81	0.90	0.60	0.66	1.20
5d	2.64	0.74	0.72	0.65	0.72	1.06	0.58	0.65	0.72
7a	0.90	1.08	1.20	0.96	0.66	1.02	0.73	0.49	0.57
7c	1.22	0.57	1.18	0.96	0.73	1.10	0.83	0.41	0.57
7e	0.89	0.72	0.62	1.06	0.79	1.00	0.61	0.54	0.79
7g	0.81	1.01	1.41	1.17	1.14	1.01	0.88	0.81	0.86

[a] For subpanel tumor cell lines see footnote [a] of Table 2.

be essential for the formation of hydrogen bonds important for the interaction with polymerases and the key enzymes of nucleic acids metabolism.

Anti-HIV-1 activity

Four compounds **6a–d** have been selected by NCI and evaluated for their effects on HIV-1 induced cytopathogenicity in a human T4-lymphocyte cell line (CEM) [30]. 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

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

N°	NCS No.	Maximum% protection	Dose (molar)	IC_{50} (molar)
6a	722237	16	2.01×10^{-6}	1.09×10^{-5}
6b	722238	57	6.34×10^{-6}	1.05×10^{-5}
6c	722227	47	6.34×10^{-6}	1.20×10^{-5}
6d	722236	45	6.34×10^{-6}	1.08×10^{-5}

Table 6. *In vitro* anti-HIV-1 activity parameters of compound **6b** (NCS 722238).

Compound	IC^{50} (Molar)	EC^{50} (Molar)	TI_{50} (IC/EC)
6b	1.05×10^{-5}	5.51×10^{-6}	1.91
AZT	$>1.00 \times 10^{-6}$	2.57×10^{-9}	3.89×10^{-2}

viral cytopathic effect. The 50% inhibitory concentration (IC_{50}), represent the toxic concentration of drug resulting in 50% growth inhibition of normal uninfected cells. The therapeutic index (TI_{50}) was determined by dividing (IC_{50}) by (EC_{50}).

Among the tested compounds, compound **6b** was confirmed to have moderate *in vitro* anti-HIV activity. It showed a good reduction of viral cytopathic effect (57%). Compounds **6c** and **6d** showed mild reduction of viral cytopathic effect by 47 and 45% respectively, while compound **6a** exhibited weak activity. It reduced the viral cytopathic effect by 16% (Table 5). The recorded IC_{50} and EC_{50} for **6b** were 1.05×10^{-5} and 5.51×10^{-6}, respectively, however the resulting therapeutic index (TI) is 1.91 which is not sufficient for further *in vivo* testing as compared with AZT (TI = $>3.89 \times 10^{-2}$, Table 6).

The aforementioned results indicated that maximum activity was observed when the the position 3 of the thiazole ring was substituted with a butyl group and position 4 of the phenyl moiety with a chlorine atom.

Antimicrobial activity

Compounds **5a–e**, **6a-d** and **7a–g** were preliminary evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (NCTC 4163) and *Bacillus subtilis* as Gram-positive bacteria and *Pseudomonas aerugenosa* (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 *Saccharamyces cercvisiae* (ATTC 9763) as examples of yeast, *Aspergillus niger* (ATTC 16404) and *Aspergillus terreus* (local isolate) as examples of true fungi. Their inhibition zones using the agar cup diffusion technique [31] were measured. Ampicillin was used as reference antibacterial, while clotrimazole was used as antifungal reference. Compounds which showed growth inhibition ≥20 mm were further evaluated for their minimal inhibitory concentration (MIC) using the two-fold serial dilution technique [32].

The results recorded in Table 7 revealed that the tested compounds exhibited promising activity towards the Gram-negative *P. aerugenosa* and *P. vulgaris*. Compound **5a** was the most active, it was four times as active as ampicillin against *P. aerugenosa* (MIC <25 μg/mL). Compound **5b** had twice the activity of ampicillin. In addition, compounds **5d**, **7c**, and **7f** were equipotent to ampicillin. Compounds **5c**, **5e**, **6b**, **6c**, **7a**, **7d**, **7e**, and **7g** showed half the activity, while compounds **6a**, **6d**, and **7a** were devoid of activity against *P. aerugenosa*.

On the other hand, compound **7c** was the most active against *P. vulgaris*, it was equipotent to ampicillin (MIC = 50 μg/mL). Compound **7b** displayed half the potency of that of ampicillin. Compounds **5a**, **5d**, **7c**, **7d**, **7f**, and **7g** displayed weak activity (one fourth the activity), while the other tested compounds were inactive. Moreover, compounds **6a–d** exhibited weak activity towards the Gram-positive *S. aureus*, compound **6c** was the active member.

Considering the antifungal activity, the tested compounds were devoid of activity except **7a**, which showed weak activity against *C. albicans*.

From the previously mentioned results it could be deduced that significant antibacterial activity was associated with the 8-(3-substituted-4-oxothiazolidin-2-ylidene) series **5a–e**. Maximum activity was achieved when the substitutent at position 3 was butyl group **5a**. Substitution at N-7 of purine nucleus greatly decreased the activity (compounds **5c**, **5d**, and **5e**). Replacement of 3-substituted-4-oxothiazolidine moity by 3,4-disubstituted-dihydrothiazol (compounds **6a–d**) decreased the activity towards the Gram-negative *P. aerugenosa* and *P. vulgaris* and slightly increased the activity towards the the Gram-positive *S. aureus*. The 5-amino-3-substituted-pyrazolyl series **7a–d** showed promising activity against the spore forming Gram-negative bacteria *P. vulgaris*. Also maximum activity was obtained when the substituent at position 3 was unsubstituted phenyl group and at N-7 of purine was hydrogen. Substitution at position 4 of phenyl group with chlorine atom slightly decreased the activity, while substitution with CH_3 group greatly decreased the activity. In addition, as previously mentioned in the anti-tumor and anti-HIV activity, substitution at N-7 of purine with methyl group greatly decreased or abolished the activity. Accordingly, it could be concluded that the pre-

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

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
5a	17	–	16	–	–	–	33	<25	14	–	20	<200
5b	–	–	14	–	–	–	32	<50	14	–	19	–
5c	–	–	13	–	–	–	21	<200	16	–	19	–
5d	16	–	–	–	–	–	22	<100	14	–	22	<200
5e	16	–	15	–	16	–	21	<200	12	–	18	–
6a	20	<200	14	–	–	–	19	–	12	–	18	–
6b	20	<200	13	–	–	–	20	<200	12	–	18	–
6c	24	<100	14	–	12	–	20	<200	12	–	19	–
6d	20	<200	15	–	–	–	18	–	12	–	18	–
7a	–	–	19	–	21	<200	20	<200	20	<200	24	<50
7b	–	–	16	–	16	–	19	–	16	–	22	<100
7c	17	–	15	–	16	–	21	<100	16	–	21	<200
5d	16	–	–	–	12	–	20	<200	12	–	20	<200
7e	17	–	–	–	–	–	21	<200	–	–	18	–
7f	18	–	12	–	–	–	22	<100	11	–	21	<200
7g	17	–	–	–	–	–	21	<200	14	–	21	<200
Ampicillin	–	5	–	5	–	100	–	100	–	10	–	50
Clotrimazole												5

Table 8. Physical and analytical data of the synthesized compounds (**4 – 7**).

N°	R	R_1	Yield [%]	M. p. [°C] Cryst. solvent	Mol. formula[a)] (Mol. Wt.)
4a	H	$(CH_2)_3CH_3$	65	>350 Dioxan	$C_{12}H_{19}N_7O_2S$ (325.39)
4b	H	$CH_2C_6H_5$	62	>350 Dioxan	$C_{15}H_{17}N_7O_2S$ (359.41)
4c	CH_3	$(CH_2)_3CH_3$	68	240-242 Dioxan	$C_{13}H_{21}N_7O_2S$ (339.42)
4d	CH_3	$CH_2C_6H_5$	73	>350 Dioxan	$C_{16}H_{19}N_7O_2S$ (373.43)
4e	CH_3	C_6H_{11} (cyclo)	84	>350 Dioxan	$C_{15}H_{23}N_7O_2S$ (365.46)
5a	H	$(CH_2)_3CH_3$	48	256 – 258 EtOH	$C_{14}H_{19}N_7O_3S$ (365.41)
5b	H	$CH_2C_6H_5$	55	253 – 255 EtOH	$C_{17}H_{17}N_7O_3S$ (399.43)
5c	CH_3	$(CH_2)_3CH_3$	79	215 – 217 EtOH	$C_{15}H_{21}N_7O_3S$ (379.44)
5d	CH_3	$CH_2C_6H_5$	77	230 – 232 EtOH	$C_{18}H_{19}N_7O_3S$ (413.46)
5e	CH_3	C_6H_{11} (cyclo)	56	252 – 254 EtOH	$C_{17}H_{23}N_7O_3S$ (405.48)
6a	H	$(CH_2)_3CH_3$	77	200 – 202 EtOH	$C_{21}H_{25}N_7O_2S.HBr$ (520.45)
6b	Cl	$(CH_2)_3CH_3$	79	196 – 198 EtOH/diethyl ether	$C_{21}H_{24}ClN_7O_2S \cdot HBr$ (554.89)
6c	H	$CH_2C_6H_5$	51	183 – 185 EtOH	$C_{24}H_{23}N_7O_2S \cdot HBr$ (554.47)
6d	Cl	$CH_2C_6H_5$	54	180 – 182 EtOH/diethyl ether	$C_{24}H_{22}ClN_7O_2S \cdot HBr$ (588.91)
7a	H	H	64	340 – 342 EtOH	$C_{16}H_{15}N_7O_2$ (337.34)
7b	H	Cl	62	348 – 350 EtOH	$C_{16}H_{14}ClN_7O_2$ (371.78)
7c	H	CH_3	57	322 – 324 EtOH	$C_{17}H_{17}N_7O_2$ (351.36)
7d	CH_3	H	87	243 – 245 EtOH	$C_{17}H_{17}N_7O_2$ (351.36)
7e	CH_3	Cl	79	265 – 267 EtOH	$C_{17}H_{16}ClN_7O_2$ (385.81)
7f	CH_3	Br	93	270 – 272 EtOH	$C_{17}H_{16}BrN_7O_2$ (430.26)
7g	CH_3	CH_3	84	287 – 289 EtOH	$C_{18}H_{19}N_7O_2$ (365.39)

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

sence of a H atom at N-7 of purine is essential for maximum biological activity.

The physical and analytical data of the synthesized compounds **4 – 7** are given in Table 8.

The authors wish to thank Dr. Hesham A. El-Enshasy, Genetic Engineering and Biotechnology Research Institute(GEBRI), Mubarak City for Scientific Research and Technology Application, Borg El-Arab, Alexandria, Egypt for performing the antibacterial and antifungal screening. The authors also wish to acknowledge the staff of the Department of Health and Human Services, National Cancer Institute, Bethesda, Maryland, USA for the anticancer and anti-HIV reports.

Experimental

Chemistry

All melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus (Sanyo) and are 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 TMS as internal standard (chemical shift in δ ppm). 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 λ 254 nm for few seconds.

8-Hydrazino-1,3-dimethyl-3,7-dihydropurine-2,6-dione ***3b***

To a well stirred ice-cooled solution of 8-diazo-1,3-dimethyl-3,7-dihydropurine-2,6-dione **2** (20.6 g, 100 mmol) in a mixture of concentrated hydrochloric acid/water (8:1) (80 mL), a solution of stannous chloride (48 g) in concentrated hydrochloric acid (60 mL) was added dropwise with stirring over a period of 1 h. The formed precipitate was filtered, dissolved in water, and neutralized with saturated solution of sodium acetate. The precipitated product was filtered, washed with water, dried, and crystallized from dimethylformamide/water, m.p. 322–332°C (reported 320°C) [21]; yield 11.2 g (53%).

8-(N-Substituted-thiocarbamoylhydrazino)-1,3-dimethyl (and 1,3,7-trimethyl)- 3,7-dihydropurine-2,6-diones ***4a–e***

To a well stirred suspension of **3a** or **3b** (5 mmol) in absolute ethanol (100 mL), the appropriate isothiocyanate (5 mmol) was added. The reaction mixture was heated under reflux for 6–12 h. The separated product was filtered while hot, washed with diethyl ether, dried, and crystallized from dioxane. (Table 8).

IR of compounds **4a–e** (KBr, cm^{-1}): 3293–3265, 3257–3208 (NH); 1690–1685 (C=O); 1650–1649, 1627–1624, 1534–1533, 1501–1498 (C=N, NH bending, C=C); 1551–1535, 1285–1278, 1075–1038, 989–947 (N-C=S amide I, II, III, IV bands).

^{1}H-NMR of compound **4a** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 0.89 (t, J = 7.2 Hz, 3H, $(CH_2)_3$-CH_3); 1.26 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 1.48 (m, 2H, $CH_2-CH_2-CH_2$-CH_3); 3.19 (s, 3H, purine-N_3-CH_3); 3.37 (s, 3H, purine-N_1-CH_3); 3.49 (m, 2H, $CH_2-(CH_2)_2-CH_3$); 8.15 (t, 1H, $NH-CH_2$, D_2O exchangeable); 9.12, 9.27 (two s, each 1H, NH-NH, D_2O exchangeable); 12.27 (s, 1H, NH, D_2O exchangeable). MS of **4b** m/z (relative abundance%): 361[M + 2] (5.5), 262 (6.2), 236 (5.2), 195 (30.2), 149 (8.8), 91 (76.6), 56 (100).

^{1}H-NMR of compound **4d** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.14 (s, 3H, purine-N_3-CH_3); 3.34 (s, 3H, purine-N_1-CH_3); 3.56 (s, 3H, purine-N_7-CH_3); 4.77 (d, 2H, NH-$CH_2-C_6H_5$); 7.21–7.32 (m, 5H, Ar-H); 8.79 (t, 1H, $NH-CH_2$, D_2O exchangeable); 9.33, 9.61 (two s, each 1H, NH-NH, D_2O exchangeable).

8-[(3-Substituted-4-oxo-thiazolidin-2-ylidene)hydrazino]-1,3-dimethyl-(and 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones ***5a–e***

To a suspension of the selected **4** (2 mmol) in absolute ethanol/dry dioxane (1:1) (20 mL), ethyl bromoacetate (0.33 g, 2 mmol) was added. The reaction mixture was heated under reflux for 4–8 hours then concentrated under reduced pressure. The precipitate formed after addition of a few drops of water was filtered, dried, and crystallized from ethanol. (Table 8).

IR of compounds **5a–e** (KBr, cm^{-1}): 3193–3100 (NH); 1727–1713 (C=O thiazolidinone); 1694–1685 (C=O purine); 1665–1638, 1620–1606, 1535–1531, 1508–1494 (C=N, NH bending, C=C); 1227–1217, 1053–1038 (C-S-C).

^{1}H-NMR of compound **5a** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 0.89 (t, J = 7.3 Hz, 3H, $(CH_2)_3-CH_3$); 1.28 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 1.55 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 3.20 (s, 3H, purine-N_3-CH_3); 3.37 (s, 3H, purine-N_1-CH_3); 3.80 (t, J = 7.2 Hz, 2H, $CH_2-(CH_2)_2-CH_3$); 4.01 (s, 2H, thiazolidinone-C_5-H_2); 10.08, 11.92 (two s, each 1H, N*H*-N, N*H* (purine), D_2O exchangeable).

^{1}H-NMR of compound **5c** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 0.88 (t, J = 7.3 Hz, 3H, $(CH_2)_3-CH_3$); 1.28 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 1.59 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 3.17 (s, 3H, purine-N_3-CH_3); 3.34 (s, 3H, purine-N_1-CH_3); 3.47 (s, 3H, purine-N_7-CH_3); 3.67 (t, J = 7.2 Hz, 2H, $CH_2-(CH_2)_2-CH_3$); 4.03 (s, 2H, thiazolidinone-C_5-H_2); 9.61 (s, 1H, N*H* – N, D_2O exchangeable). MS of **5c** m/z (relative abundance%): 379 [M^+] (43.7), 208 (90.2), 151 (15.0), 94 (17.1), 82 (100).

8-[(3,4-Disubstituted-2,3-dihydrothiazol-2-ylidene)hydrazino]-1,3,7-trimethyl-3,7-dihydropurine-2,6-diones hydrobromides ***6a-d***

A mixture of **4c** or **4d** (2 mmol) and the appropriate phenacyl bromide (2 mmol) in absolute ethanol (20 mL) was heated under reflux for 30 minutes. The reaction mixture was then concentrated and left to cool to room temperature. The separated crystalline product was filtered, dried, and recrystallized from the proper solvent. (Table 8).

IR of compounds **6a–d** (KBr, cm^{-1}): 3288-3095 (NH); 1703–1692 (C=O); 1664–1646, 1618–1608, 1599–1534 (C=N, NH bending, C=C); 1222–1212, 1092–1035 (C-S-C).

^{1}H-NMR of compound **6b** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 0.71 (dist. t, 3H, $(CH_2)_3-CH_3$); 1.15 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 1.59 (m, 2H, $CH_2-CH_2-CH_2-CH_3$); 3.21 (s, 3H, purine-N_3-CH_3); 3.35 (s, 3H, purine-N_1-CH_3); 3.81 (s, 3H, purine-N_7-CH_3); 4.00 (dist. t, 2H, $CH_2-(CH_2)_2-CH_3$); 7.16 (s, 1H, thiazoline-C_5-H); 7.40–7.66 (m, 4H, Ar-*H*); 10.53 (s, 1H, N*H*, D_2O exchangeable).

^{1}H-NMR of compound **6c** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.19 (s, 3H, purine-N_3-CH_3); 3.39 (s, 3H, purine-N_1-CH_3); 3.49 (s, 3H, purine-N_7-CH_3); 5.19 (s, 2H, $CH_2-C_6H_5$); 6.89 (d, 2H, Ar-$C_{2,6}-H$ of benzyl group); 7.09 (s, 1H, thiazoline-C_5-H); 7.28–7.50 (m, 8H, Ar-*H*); 9.74 (s, 1H, N*H*, D_2O exchangeable).

MS of **6d** m/z (relative abundance%): 507 [M^+] (1.2), 434 (1.0), 381 (1.39), 255 (2.47), 201 (7.28), 153 (23.1), 109 (100), 58 (89.7).

8-(5-Amino-3-arylpyrazol-1-yl)-1,3-dimethyl (and 1,3,7-trimethyl)-3,7-dihydropurine-2,6-diones ***7a-g***

To a solution of **3a** or **3b** (2 mmol) in ethanol/acetic acid (4:1) (10 mL), the appropiate ω-cyanoacetophenone (2 mmol) was added. The reaction mixture was heated under reflux for 6 h and left to cool to room temperature. The separated crystalline product was filtered, dried, and recrystallized from ethanol.

IR of compounds **7a–g** (KBr, cm^{-1}): 3423–3343, 3327–3265, 3187–3179 (NH_2, NH); 1710–1696 (C=O); 1665–1647, 1548–1540, 1513–11507 (C=N, NH bending, C=C).

^{1}H-NMR of compound **7b** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.27 (s, 3H, purine-N_3-CH_3); 3.51 (s, 3H, purine-N_1-CH_3); 5.90 (s, 1H, pyrazole-C_4-H); 6.75 (s, 2H, NH_2, D_2O exchangeable); 7.50 (d, J = 8 Hz, 2H, Ar-$C_{2,6}-H$); 7.94 (d, J = 8 Hz, 2H, Ar-$C_{3,5}-H$); 13.60 (s, 1H, N*H*, D_2O exchangeable).

^{1}H-NMR of compound **7c** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 2.32 (s, 3H, 4-C*H*$_3$ – C_6H_4); 3.27 (s, 3H, purine-N_3 – C*H*$_3$); 3.48 (s, 3H, purine-N_1 – C*H*$_3$); 5.82 (s, 1H, pyrazole-C_4 – *H*); 6.70 (s, 2H, N*H*$_2$, D_2O exchangeable); 7.22 (d, J = 8 Hz, 2H, Ar-$C_{3,5}$ – *H*); 7.77 (d, J = 8 Hz, 2H, Ar-$C_{2,6}$ – *H*); 12.06 (s, 1H, NH, D_2O exchangeable). MS of **7c** m/z (relative abundance%): 351 [M$^+$] (100), 265 (12.3), 240 (10.4), 184 (19.1), 142 (25.6), 115 (40.6), 82 (44.5).

^{1}H-NMR of compound **7d** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.18 (s, 3H, purine-N_3 – C*H*$_3$); 3.39 (s, 3H, purine-N_1 – C*H*$_3$); 4.10 (s, 3H, purine-N_7-C*H*$_3$); 5.85 (s, 1H, pyrazole-C_4 – *H*); 6.47 (s, 2H, N*H*$_2$, D_2O exchangeable); 7.31 – 7.40 (m, 3H, Ar-$C_{3,4,5}$ – *H*); 7.76 (d, 2H, Ar-$C_{2,6}$ – *H*).

^{1}H-NMR of compound **7e** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.17 (s, 3H, purine-N_3 – C*H*$_3$); 3.33 (s, 3H, purine-N_1 – C*H*$_3$); 4.08 (s, 3H, purine-N_7 – C*H*$_3$); 5.86 (s, 1H, pyrazole-C_4 – *H*); 6.50 (s, 2H, N*H*$_2$, D_2O exchangeable); 7.40 (d, J = 8 Hz, 2H, Ar-$C_{2,6}$ – *H*); 7.73 (d, J = 8 Hz, 2H, Ar-$C_{3,5}$ – *H*). MS of **7e** m/z (relative abundance %): 387 [M + 2] (37.5), 385 [M$^+$] (54.5), 343 (2.5), 272 (3.0), 247 (5.9), 178 (10.9), 136 (48.6), 111 (15.3), 67 (100).

^{1}H-NMR of compound **7f** (DMSO-d_6, δ ppm, JNM-LA 400 FT): 3.20 (s, 3H, purine-N_3 – C*H*$_3$); 3.41 (s, 3H, purine-N_1 – C*H*$_3$); 4.09 (s, 3H, purine-N_7 – C*H*$_3$); 5.89 (s, 1H, pyrazole-C_4 – *H*); 6.49 (s, 2H, N*H*$_2$, D_2O exchangeable); 7.56 (d, J = 8 Hz, 2H, Ar-$C_{2,6}$ – *H*); 7.71 (d, J = 8 Hz, 2H, Ar-$C_{3,5}$ – *H*).

Biological evaluation

Anticancer activity

The prepared compounds were tested for their *in vitro* anticancer activity against 60 human tumor cell lines, derived from nine clinically isolated 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, ten-fold dilution, 48 h continuous drug exposure protocol was used and a sulforodamine B (SRB) protein assay was used to estimate cell viability or growth [27].

In vitro anti-HIV-1 activity

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 [30]. The assay involved the killing of T_4 lymphocytes by HIV. The 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}. 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 six 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.

Antimicrobial activity

Inhibition zones measurement

The compounds were dissolved in DMSO in a concentration of 1 mg/mL by the agar cup diffusion technique [31] using a 1 mg/mL solution in DMSO. Each 100 mL of sterile molten agar (at 45°C) received 1 mL of 6 h broth, 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 1 mg/mL solution of the test compounds. The plates were then incubated at 37°C for 24 h or 48 h for fungi. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference. The resulting inhibition zones are recorded (Table 7).

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentrations (MIC) of the most active compounds were measured using the two fold serial broth dilution method [32]. The test organisms were grown in their suitable broth for 24 hours for bacteria and 48 hours for fungi at 37°C. Two-fold serial dilutions of the test compounds solution were prepared using the suitable broth to obtain concentrations 200, 100, 50, 25, and 12.5 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 hours. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the tested compounds are listed in Table 7.

References

[1] S. Herber, J. S. Lilleyman, J. Maddocks, *Br. J. Cancer* **1982**, *46*, 138 – 141.

[2] J. V. Simone, *Br. J. Haematol.* **1980**, *45*, 1 – 4.

[3] A. Gangjee, A. Vasudevan, S. F. Queener, *J. Med. Chem.* **1997**, *40*, 3032 – 3039.

[4] E. Lech-Maranda, A. Korycka, T. Robak, *Mini Rev Med Chem.* **2006**, *6*, 575 – 581.

[5] G. R. Revankar, N. B. Hanna, N. Imamura, A. F. Lewis, *et al*., *J. Med. Chem.* **1990**, *33*, 121 – 128.

[6] K. Ramasamy, N. Imamura, N. B. Hanna, R. A. Finch, *et al*., *J. Med. Chem.* **1990**, *33*, 1220 – 1225.

[7] T. L. Avery, R. A. Finch, K. M.Vasquez, S. Radparver, *et al*., *Cancer Res.* **1990**, *50*, 2625 – 2630.

[8] P. W. K. Woo, C. R. Kostlan, J. C. Sircar, M. K. Dong, R. B. Gilbertsen, *J. Med. Chem.* **1992**, *35*, 1451 – 1457.

[9] M. Steurer, G. Pall, S. Richards, G. Schwarzer, *et al*., *Cancer Treat Rev.* **2006**, *32*, 377 – 389.

[10] C. McLaren, R. Datema, C. A. Knupp, R. A. Buroker, *Antivir. Chem. Chemother*. **1991**, *2*, 321 – 328.

[11] V. A. Johnson, D. P. Merril, J. A. Videler, T. C. Chou, *et al*., *J. Infect. Dis.* **1991**, *164*, 646 – 655.

[12] N. Valiaeva, J. R. Beadle, K. A. Aldern, J. Trahan, K. Y. Hostetler, *Antiviral Res.* **2006**, *72*, 10 – 19.

[13] S. Raic-Malic, A. Hergold-Brudic, A. Nagl, M. Grdisa, *et al*., *J. Med Chem.* **1999**, *42*, 2673 – 2678.

[14] T. Sekiyama, S. Hatsuya, Y. Tanaka, M. Uchiyama, *et al.*, *J. Med Chem.* **1998**, *41*, 1284 – 1298.

[15] K. Lee, Y. Choi, E. Gullen, S. Schluete-Wirtz, *et al.*, *J. Med. Chem.* **1999**, *42*, 1320 – 1328.

[16] Y. Li, L. Fu, H. Yeo, J. L. Zhu, *et al.*, *Antivir. Chem. Chemother.* **2005**, *16*, 193 – 201.

[17] E. Kmonickova, P. Potmesil, A. Holy, Z. Zidek, *Eur. J. Pharmacol.* **2006**, *530*, 179 – 187.

[18] I. G. Zinchenko, A. A. Kremzer, Y. V. Strokin, A. N. Krasovskii, P. N. Steblyuk, *Farm. Zh. (Kiev)* **1987**, *3*, 39; *Chem. Abstr*. **1988**, *108*, 150134j.

[19] J. Piosik, A. Gwizdek-Wisniewska, K. Ulanowska, J. Ochocinski, A. Czyz, G. Wegrzyn, *Acta Biochim. Pol*. **2005**, *52*, 923 – 926.

[20] M. Lazarczyk, T. Grzela, J. Niderla, M. A. Lazarczyk, *et al*., *Oncol Rep.* **2004**, *11*, 1121 – 1125.

[21] H. Priewe, A. Poljak, *Chem. Ber.* **1955**, *88*, 1932; *C. A.* **1956**; *50*, 12067d.

[22] J. W. Jones, R. K. Robins, *J. Am. Chem. Soc*. **1960**, *82*, 3773 – 3779.

[23] N. Ergenc, G. Capan, *Farmaco* **1994**, *49*, 133 – 135.

[24] F. A. Ashour, S. A-Almazroa, *Farmaco* **1990**, *45*, 1207 – 1218.

[25] O. Ates, A. Salman, N. Cesur, G. Otuk, *Pharmazie* **1993**, *48*, 143 – 144.

[26] S. A. El-Hawash, N. S. Habib, N. H. Fanaki, *Pharmazie* **1999**, *54*, 808 – 813.

[27] M. R. Grever, S. A. Schepartz, B. A. Chabner, *Semin. Oncol*. **1992**, *19*, 622 – 638.

[28] M. R. Boyed, K. D. Paull, *Drug Dev. Res.* **1995**, *34*, 91 – 109.

[29] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, *et al.*, *J. Natl. Cancer Inst.* **1991**, *83*, 757 – 766.

[30] O. W. Weislow, R. Kiser, D. Fine, J. Bader, *et al.*, *J. Natl. Cancer Inst.* **1989**, *81*, 577 – 586.

[31] S. R. Jain, A. Kar, *Planta Med.* **1971**, *20*, 118 – 123.

[32] A. C. Scott in *Practical Medical Microbiology* (Eds.: J. G. Colle, J. P. Duguid, A. G. Fraser, B. P. Marmion, M. MacCartney), Churchil Livingstone, 13th edition, **1989**, *2*, pp. 161 – 181.

