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Original article

Synthesis, antitumor and antiviral properties of some 1,2,4-triazole derivatives

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Abstract

A series of 1,5-dialkyl-1,2,4-triazole derivatives of acetic acid alkylidene hydrazides **8–12**, the acid **13**, 1,5-dialkyl-3-(5-mercapto-4-*N*-aryl-1*H*-[1,2,4]-triazol-3-ylmethylene)-1*H*-[1,2,4] triazoles **14–16**, their 1,3,4-oxadiazole analogues **17–21**, as well as the 1,2,4-triazolo-indoles **25** and **27** were prepared. The *Z/E* conformations of some acetic acid alkylidene derivatives were studied by NMR spectroscopy. Most of the target compounds were evaluated in a series of human cancer cell in cultures and none have shown activity except **25** which exhibited remarkable activity against nine cancer types. No in vitro antiviral activity against HIV-1, HIV-2, HSV-1, HSV-2, SV, CV-B4, RSV, P3V, RV, SinV, PTV has been found for all the synthesized compounds.

1. Introduction

The 1*H*-1,2,4-triazole compounds possess important pharmacological activities such antifungal and antiviral activities. Examples of such compounds bearing the 1,2,4-triazole residues are fluconazole **1** [1], the powerful azole antifungal agent as well as the potent antiviral *N*-nucleoside ribavirin [2]. Furthermore, various 1,2,4-triazole derivatives have been reported as fungicidal [3], insecticidal [4], antimicrobial [5], and some showed antitumor activity [6], as well as anticonvulsants [7], antidepressants [8] and plant growth regulator anticoagulants [9]. Other laboratories reported the same biological activity of the triazole family [10–12]. In connection with our work on 1,2,4-triazole having hydrazide and oxadiazole moieties, we demonstrate here some potency of the reported hydrazide derivatives e.g. the quinolino-hydrazide derivative as phosphodiestrease enzyme (PDE IV) inhibitor [13] for the treatment of asthma [14], as well as steroidal antiinflammatory drug and β-agonist [15]. However, many drugs of benzimidazoles and theophylline containing the hydrazide moieties exhibited remarkable potential activity, such as anthelminitics [16,17] and monoamine oxidase inhibitors [18], respectively. On the other hand, some 1,2,4-oxadiazole derivatives, such as 5-substituted 1,2,4-oxadiazole-2-thiones [19], are known to possess remarkable activity against *Mycobacterium tuberculosis*. Recently, new 2,5-disubstituted 1,3,4-oxadiazoles [20–22] have been synthesized as possible insecticidal and antibiotic mimetics, while oxadiazolidinethiones [23] were prepared as potent antibactericidal and/or antifungicidal or antimicrobial agents. In addition to the biological importance of oxadiazoles, some indoles have been reported as chemotherapeutic agent e.g. the antibiotic rebeccamycine **2**, which exhibited in vivo antitumor activity against P388 and L1210 leukemias and B16 melanoma in mice [24], as well as the vincristine and vinblastine [25]. On the other hand, Delaviridine (Rescriptor®) **3** [26] is an indole analogue, considered as one of the non-nucleoside reverse transcriptase inhibitors and approved as a marketing anti-HIV drug. Our recent work is concerned with the synthesis of different 1,2,4-triazole compounds such as: 1,2,4-triazole-*C*-nucleosides [27,28], acyclic *C*-nucleosides [29] and homo-C-nucleosides [29], pyrimidines [30], D-*manno*-pentitol-1-yl-1,2,4-triazoles [31], *N*-alkylphthalimides [32], 1*H*-indoles [33], quinolones [33], benzotriazoles [34], 3′-triazolo-thymidines [35], and piperazines [36] from cycloaddition of the reactive intermediates 1-(chloroalkyl)-1-aza-2-azoniaallenes with the correspond-

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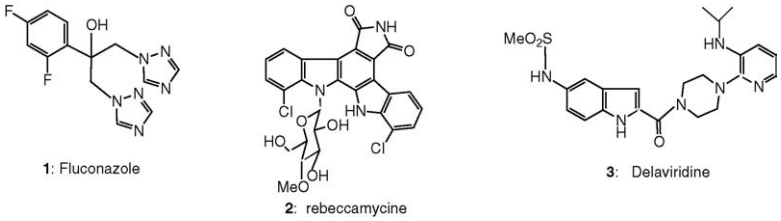
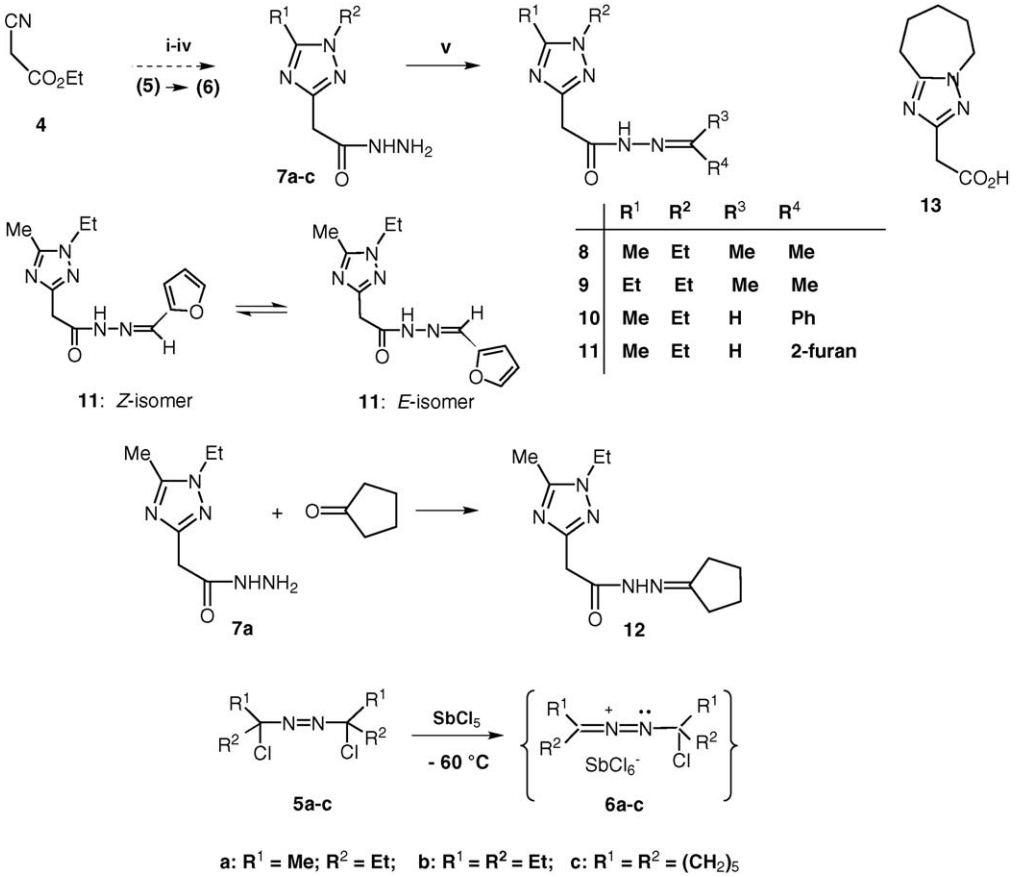
ing nitriles. The pharmacological activity of such substituted 1,2,4-triazole compounds prompted us to synthesize a series of new potentially active groups bearing the 1,2,4-triazole residues such as hydrazides, 1,3,4-oxadiazole-2-thione, mercapto-1,2,4-triazole and benzylindole, as potential antiviral or antitumor agents.



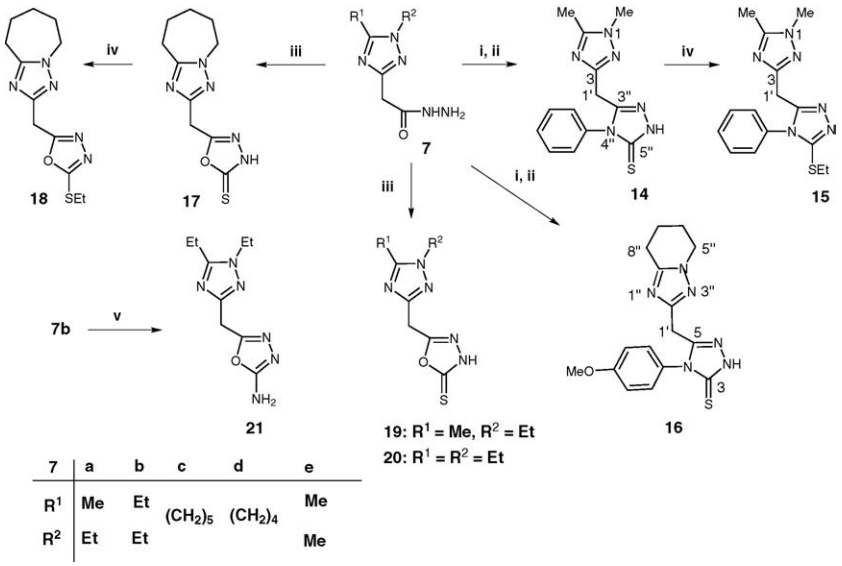
2. Chemistry

In the present study, the reactive intermediates **6a–c**, prepared in situ from the dichloride **5a–c**, were reacted via the cycloaddition reaction with ethyl cyanoacetate **4** to give, after spontaneous rearrangement [37,38], the triazole-hydrazides **7a–c** [34]. These compounds were used as starting materials for the synthesis of the alkylidene derivatives **8–13**, by heating with the appropriate aldehydes or ketones in EtOH for 4–5 h in 70–83% (Scheme 1). The structures of the newly compounds were established by their ^{1}H, ^{13}C NMR and by mass spectra. Interestingly, the ^{1}H NMR spectra of **8** and **9** showed one isomer only, meanwhile compounds **10** and **11** characterized by the presence of *Z*- and *E*-isomers, due to the different groups around the unsaturated center. Compound **11** was selected for further study by ^{1}H NMR measurements at different temperatures to establish the effect of the free roration around the double bond between 30 and 130 °C. At 30 °C, the two *Z*/*E* isomers has been clearly confirmed, when NH signal appeared as two half-singlets at δ 11.52/11.36, representing each a 1/2H, and the =C*H*furan as two half-singlets at δ 8.11/7.90, as well as the CH_2-1′ at δ 3.93/3.50 as two half-singlets. At higher temperature ca. 120 °C, these obesrvation is changed by the ^{1}H NMR spectrum, which shows one isomer, proved from the signals of NH, =C*H*furan and CH_2-1′, oriented as three singlets at δ



	R1	R2	R3	R4
8	Me	Et	Me	Me
9	Et	Et	Me	Me
10	Me	Et	H	Ph
11	Me	Et	H	2-furan

Scheme 1. *Conditions and reagents:* (i) **5a-c,** (ii) $SbCl_5$; CH_2Cl_2, – 60 °C to 23 °C, 7 h, CH_2Cl_2; (iii) aq. $NaHCO_3$, NH_3, MeCN, 0 °C, 2 h; (iv) NH_2NH_2; (v) RCHO or RCOR, EtOH.



Scheme 2. *Conditions and reagents:* (i) *p*-X-PhSCN, reflux, 4 h; (ii) 5% NaOH, reflux, 3 h, then neutraliz. HCl; (iii) CS_2, EtOH-KOH, reflux, 8 h; (iv) EtI, THF-NaOH, 0 °C, 1 h; (v) CNBr, EtOH, 70 °C, 3 h.

10.93, 8.06, and 4.00, respectively (Scheme 1). The same observation was recorded by the ^{13}C NMR spectra at 30 and 120 °C (see Section 4). The two isomers in the ^1H NMR spectrum of **12** is explained in term of the endo and exo forms of the cyclopentane ring.

Next, the hydrazides **7a–e** were used in synthesis of different 1,2,4-triazole-3-thiones and 1,3,4-oxadiazole-2-thiones. Thus, treatment of **7e** with phenyl isothiocyanate in boiling temperature for 4 h gave the thiosemicarbazide solid, which separated and boiled directly with 5% NaOH for 3 h to provide, after neutralization with dil. HCl, **14** (67%). Similarly, treatment of **7d** with 4-methoxyphenylisocyanate afforded **16** (62%).

By applying the reported procedure [39], the 1,3,4-oxadiazole derivatives **17, 19**, and **20** were prepared in 83%, 77%, and 79% yields, respectively, from boiling of a solution of **5c, 5a**, and **5b** in EtOH containing KOH and CS_2 for 8 h, followed by neutralization with dil. HCl.

Treatment of **7b** with bromocyanide (BrCN) at 70 °C for 3 h afforded the amino compound **21** (76%). The structures of all the newly synthesized compounds were confirmed by the ^1H-, ^{13}C NMR and mass spectra. Compounds **14** and **16** were identified by the homo- and heteronuclear spectra, as well as from comparison to the 1,2,4-triazolo-azipene derivatives prepared previously [35]. CH_2-1′ (δ_H 3.81, 3.87, respectively) showed two $^2J_{C,H}$ correlations: one to C-3 (δ_C 155.7), C-3″ (δ_C 149.5) of **14** and the other to C-2″ (δ_C 156.9), C-5 (δ_C 149.3) of **16**. Gradient selected HMQC [40] and HMBC [41] spectra of **16** allowed via $^2J_{C,H}$ and $^3J_{C,H}$ couplings the assignment of the CH_2-5″–CH_2-9″. In a similar spectral analysis, the structures of the 1,3,4-oxadiazoles derivatives **17–21** have been confirmed. Compound **17** was selected for HSQC [42], homo- and heteronuclear NMR study. CH_2-1′ in the HSQC appeared as singlet at δ_H 4.32 which cross-linked to C-1′ at δ_C 25.6, while CH_2-5″–CH_2-9″ at δ_H 4.52, 1.77, 1.84, 1.86, and 3.00 were cross-linked to C-5″–C-9″ at δ_C 51.4, 29.1, 38.7, 24.4 and 26.8, respectively. Gradient selected HMBC spectrum allowed the identification of C-10″ at δ_C 157.2 from the $^2J_{C,H}$ and $^3J_{C,H}$ correlations to CH_2-9″ at δ_C 3.00 and CH_2-5″ at δ_H 4.52. C=S was appeared at higher field at δ_C 178.0, while C-5 of oxathiadiazole ring oriented at δ_C 158.9. The structure of **21** was assigned from the ^1H NMR and mass spectra [FABMS *m/z* 245 (MNa^+)]. The amino group and CH_2-1′ were appeared as broad singlet and singlet at δ 6.79 and 3.97, respectively (Scheme 2).

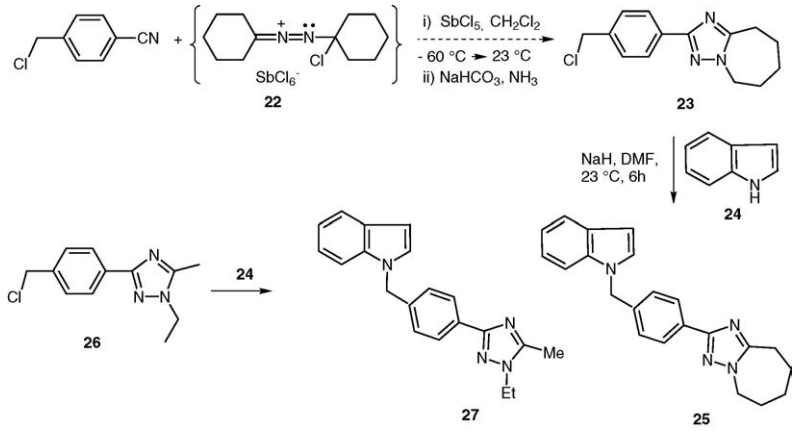
Our efforts in searching for biologically active candidates led us to the recent synthesized triazolo-indoles **25** and **27** [33] as interesting products for the antiviral and antitumor evaluation, whereby **25** showed a remarkable activity against different cancer cell lines. These compounds were prepared from condensation of the indole **24** with the *p*-chlorobenzyl triazole derivatives **23** and **26** in the presence of the hydride ions, respectively (Scheme 3).

3. Results and discussion

3.1. Antitumor activity

Compounds **12, 14, 16–20, 25** and **27** were evaluated for the antitumor activity according to NCI in vitro protocols. They were assayed in vitro against three cancer types: breast, lung and central nervous system (CNS) cancers, while **25** was screened against a panel consisting of 60 human tumor cell lines, derived from nine cancer types (leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers, at five, 10-fold dilutions from a maximum of 10^{-4} M. The results are displayed in Table 1.

Only compound **25** showed marked activity against colon (HCC-2998), and melanoma (UACC-257) cancers, with low percentage growth of $\log_{10}$ concentration = –2.0 at 10^{-5} and –83 at 10^{-4} M, respectively, since the negative value indicates



Scheme 3.

cells killed at the mentioned concentrations. In addition, the same compound exhibited remarkable activity against individual cell lines e.g., melanoma (LOX IMVI), ovarian (OVCAR-3); prostate (PC-3) and breast (NCI/ADR-RES) cancers with $\log_{10}$ values of –69, –62, –64, and –68, respectively.

In conclusion, compound **25** shows activity against every type of cancer cell line tested, but the anticancer activity is moderate or weak (GI ≥ 10^{-4} to 10^{-5} M), in comparison with the lead series of the anticancer agents, vincristine and vinblastine [25].

3.2. Antiviral activity

Compounds **7c,e, 8–11, 14–19** and **27** were evaluated for their anti-HIV activity in vitro using III_B strain for HIV-1 and the ROD strain for HIV-2, and monitored by the inhibition of the virus-induced cytopathic effect in MT-4 cells. The results are shown in Table 2.

None of these compounds were found to inhibit HIV-1 or HIV-2 replication, in vitro, at EC_{50} lower than the CC_{50}, in comparison to the antiviral agent delviridine. Compound **27** had IC_{50} value of 0.438 μg/ml, with CC_{50} 0.92 μg/ml and SI value < 1.0, which represent no selective anti-HIV activity could be witnessed.

All the above compounds were also evaluated against other viruses e.g. herpes simplex viruses [HSV-1 (KOS strain), HSV-2 (G strain)]; vaccinia virus; in E_6SM cell cultures; vesicular stomatitis virus; Coxsackie virus B4; respiratory syncytical virus, in HeLa cell cultures; parainfluenza-3 virus; reovirus-1; Sindbis virus; Punta Toro virus, in Vero cell cultures. No activity was shown for any of these compounds at non-toxic concentrations, since the minimum inhibitory concentration required to reduce the virus-induced cytopathogenicity by 50% was higher than 80 or 400 μg/ml, in comparison to the known antiviral drugs ribavirin [43], acyclovir (Zovirax®) [44], ganciclovir (Cymevene®) [45,46], and *(S)*-DHPA) [47] (Tables 3 and 4).

Table 1
In vitro model primary anticancer data [a] for some of 1,2,4-triazole derivatives at concentration (10^{-4} M)

Compound	Growth percentages (GP)									Activity
	I	II	III	IV	V	VI	VII	VIII	IX	
12	–	107	–	101	–	–	–	–	108	Inactive
14	–	104	–	160	–	–	–	–	116	Inactive
16	–	86	–	101	–	–	–	–	98	Inactive
17	–	45	–	55	–	–	–	–	87	Inactive
18	–	56	–	89	–	–	–	–	97	Inactive
19	–	83	–	110	–	–	–	–	107	Inactive
20	–	42	–	79	–	–	–	–	80	Inactive
25	–31[b,c]	–11[e]	–63[g]	–39[k]	–69[n]	–62[s]	–58[t]	–64[w]	–68[x]	Active
	–26[d]	–3[f]	–2[h,i]	–34[l]	–20[o]		–24[u]		–28[y]	Active
			–19[j]	–43[m]	–12[p]		–1[v]		–61[z]	Active
					–83[q]					Active
					–35[r]					Active
27	–	39	–	67	–	–	–	–	103	Inactive

I. leukemia ([c] MOLT-4, [d] RPMI-8226); II. non-small cell lung ([e] HOP-62, [f] HOP-92); III. colon cancer ([g] COLO 205, [h] HCC-2998 at 10^{-4} M, [i] HCC-2998 at 10^{-5} M, [j] KM12); IV. CNS cancer ([k] SF-295, [l] SF-539, [m] SNB75); V. melanoma ([n] LOX IMVI, [o] MALM-3M, [p] SK-MEL-28, [q] UACC-257, [r] UACC-62); VI. Ovarian cancer ([s] OVCAR-3); VII. renal cancer ([t] CAKI-1, [u] SN12C, [v] TK-10); VIII. Prostate cancer ([w] PC-3); IX. Breast cancer ([x] NCI/ADR-RES, [y] MDA-MB-231/ATCC, [z] MDA-MB-435).

[a] Results for each test agent are reported as the percentage growth of the treated cell compared to the untreated control cells.

[b] Negative number indicates cell kill at a concentration 10^{-4} or 10^{-5} M.

Table 2
In vitro anti-HIV-1 [a] and HIV-2 [b] activity for some 1,2,4-triazole compounds

Compound	Strain	IC_{50} (μg/ml) [c]	av.CC_{50} (μg/ml) [d]	SI [e]
7c	IIB	>125	>125	X 1
	ROD	>125	>125	X 1
7e	III_B	>102	98.4	<1
	ROD	>94.8	–	–
8	III_B	>95.1	–	–
	ROD	>108	107.37	<1
9	III_B	>117	112.38	<1
	ROD	>98.5	112.38	<1
10	III_B	>104	–	–
	ROD	>125	>125	X 1
11	III_B	>102	103.67	<1
	ROD	>89	103.67	<1
14	III_B	>59.3	–	–
	ROD	>60.8	–	–
16	III_B	>97.4	105.2	<1
	ROD	113	–	–
17	III_B	>66.6	68.78	<1
	ROD	>68	–	–
19	III_B	>58.3	69.38	<1
	ROD	>63.4	–	–
27	III_B	>0.48	0.92	<1
	ROD	0.436	0.92	<1
Delviridine [26]	III_B	>10 8 (PBMC) (EC_{50} = 0.05 μM, MT4)		–

[a] Anti-HIV-1 activity measured with strain IIIB.
[b] Anti-HIV-2 activity measured with strain ROD.
[c] Inhibitory minimum concentration of compound required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV.
[d] Cytotoxic concentration of compound required to reduce the viability of mock-infected MT-4 cells by 50%.
[e] Selectivity index: ratio of CC_{50}/IC_{50}.

Although compound **27** showed activity against HIV-1 and HIV-2 at >0.48, and 0.436 μg/ml, respectively, but the value of SI < 1.0 is indicating of no considerable selective cytotoxicity. Similarly, **7c** exhibited activity against Parainfluenza-3 virus, Reovirus-1, Sindbis virus and Punta Toro virus at 16 μg/ml, with SI < 1.0 (data not reported), and again revealed no selective activity against the mentioned viruses as well.

4. Experimental

Melting points are uncorrected. NMR spectra at 300 and 600 MHz (^1H) and at 150.91 MHz (^{13}C) with TMS as internal standard and on δ scale in ppm. The signal assignments for protons were verified by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by ^1H-^{13}C COSY or HMQC experiments. EI and FAB mass spectra were measured on an MAT8200 mass spectrometer using 3-nitrophenol (NBOH) or glycerol as matrix.

*4.1. General procedure for the preparation of 1,5-dialkyl-1,2,4-triazole derivatives of acetic acid hydrazides (**7a–c**)*

These compounds were prepared according to the procedure reported in the literature [34] from the cycloaddition of ethyl cynoacetate **4** (3.0 mmol) and the dichlorides **5a–c** (4.0 mmol) in dry CH_2Cl_2, in the presence of $SbCl_5$ (4.0 mmol) at –60 °C. After working-up, the isolated products (5.46 mmol) were treated with hydrated hydrazine (25 mmol) at 23 °C for 72 h in EtOH. The crude products were recrystallized from EtOH to give **7a–c**, as yellow crystals.

4.2. General procedure for the preparation of 1,5-dialkyl-1,2,4-triazole derivatives of acetic acid alkylidene hydrazides

A suspension of **7** (5.0 mmol) in EtOH (30 ml) and the appropriate aldehyde or ketone (5.20 mmol) was heated under reflux for 4–6 h. After cooling, the product was collected and recrystallized from EtOH to afford the desired product.

*4.2.1. (1-Ethyl-5-methyl-1H-[1,2,4]triazol-3-yl)-acetic acid isopropylidene hydrazide (**8**)*

From **7a** (0.92 g). Yield: 0.95 g, 85%; m.p. 117–121 °C. ^1H NMR ($CDCl_3$): δ 13.50 (s, 1H, NH); 4.03 (q, 2H, J = 7.5 Hz, N-CH_2CH_3); 3.73 (s, 2H, CH_2-1′); 2.38 (s, 3H, C_5-Me); 2.03, 1.88 (2 × s, 6H, 2 × C=Me_2); 1.35 (s, 3H,

Table 3
In vitro antiviral activity against different viruses other than HIV in HeLa cell cultures

Compound [a]	Minimum inhibitory concentration [b] (MIC) (μg/ml)							CC_{50} [c] (μg/ml)
	I	II	III	IV	V	VI	VII	
14	400	400	400	400	80	80	80	400
15	400	80	80	80	80	400	400	400
16	80	80	80	80	80	80	80	400
27	80	80	80	80	80	80	80	400
Ribavirin	400	400	400	400	80	48	48	400
Acyclovir	0.128	0.64	400	400	400	80	–	400
Ganciclovir	0.0192	0.032	100	100	100	2.4	–	100

I. Herpes simplex virus-1 (KOS strain); II. Herpes simplex virus-2 (G strain); III. Vaccinia virus; IV. Vesicular stomatitis virus; V. Herpes simplex virus-1 TK (KOS ACV); VI. Coxsackie virus B4; VII. Respiratory syncytical virus; VIII. Parainfluenza-3 virus; IX. Reovirus-1; X. Sindbis virus; XI. Punta Toro virus.
[a] Compounds **7c, 7e, 8, 9, 11, 19** have MIC and CC_{50} > 400 μg/ml for all the mentioned viruses.
[b] Required to cause a microscopically detectable alteration of normal cell morphology.
[c] Required to reduce virus-induced cytopathogenecity by 50%.

Table 3
Continue

Compound	EC_{50} (μg/ml)				CC_{50} (μg/ml)
	VIII	IX	X	XI	
7c	16	>16	>16	>16	80
7e	>80	>80	>80	>80	400
8	>80	>80	>80	>80	400
9	>80	>80	>80	>80	400
11	>80	>80	>80	>80	400
14	>80	>80	>80	>80	400
16	>400	>400	>400	>400	>400
17	>80	>80	>80	>80	≥08
19	>400	>400	>400	>400	≥004
27	>80	>80	>80	>80	≥08
Ribavirin	80	48	>400	48	>400
(S)-DHPA	240	240	>400	>400	>400

VIII. Parainfluenza-3 virus; IX. Reovirus-1; X. Sindbis virus; XI. Punta Toro virus.

N-CH_2*CH_3*); ^{13}C NMR ($CDCl_3$): δ 163.7 (C=O); 156.8 (C-3); 154.4 (C-5); 151.6 (Me_2*C*=N); 43.3 (N-*CH_2*CH_3); 34.9 (CH_2-1′); 25.2, 17.2 (*Me_2*C=N); 14.9 (N-CH_2*CH_3*); 11.7 (C_5-*Me*). Anal. cacld for $C_{10}H_{17}N_5O$ (223.3): C, 53.79; H, 7.67; N; 31.37. Found: C, 53.58; H, 7.68; N, 31.19. MS: *m/z* (EI) 223 $(M)^+$.

4.2.2. (1,5-Diethyl-1H-[1,2,4]triazol-3-yl)-acetic acid isopropylidene hydrazide ***(9)***

From **7b** (0.97 g). Yield: 0.95 g, 80%; m.p. 165–168 °C. ^{1}H NMR ($CDCl_3$): δ 10.84 (s, 1H, NH); 4.04 (q, 2H, *J* = 7.2 Hz, N-*CH_2*CH_3); 3.74 (s, 2H, CH_2-1′); 2.69 (q, 2H, *J* = 7.5 Hz, C_5-*CH_2*CH_3); 2.04, 1.90 (2 × s, 6H, 2 × C=Me_2); 1.36, 1.28 (2 × t, 6H, *J* = 7.5 Hz, CH_2*CH_3*); ^{13}C NMR ($CDCl_3$): δ 163.8 (C=O); 156.6 (C-3); 156.2 (C-5); 155.2 (Me_2*C*=N); 42.9 (N-*CH_2*CH_3); 34.8 (CH_2-1′); 25.1, 19.0 (*Me_2*C=N); 15.0 (N-CH_2*CH_3*); 11.6 (C_5-CH_2*CH_3*). Anal. cacld for $C_{11}H_{19}N_5O$ (237.3): C, 55.68; H, 8.07; N; 29.51. Found: C, 55.49; H, 8.01; N, 29.34. MS: *m/z* (EI) 237 $(M)^+$.

4.2.3. (1-Ethyl-5-methyl-1H-[1,2,4]triazol-3-yl)-acetic acid benzylidene hydrazide ***(10)***

From **7a** (0.92 g). Yield: 1.03 g, 76%; m.p. 152–155 °C. ^{1}H NMR ($CDCl_3$): δ 13.78 (s, 1H, NH); 7.40 (t, 3H, *J* = 3.0 Hz, Ar); 7.22–7.19 (m, 2H, Ar); 3.90 (q, 2H, *J* = 7.5 Hz, N-*CH_2*CH_3); 3.88 (s, 2H, CH_2-1′); 3.38 (br s, 1H, 1H, =C*H*Ar); 2.57 (s, 3H, C_5-Me); 1.10 (s, 3H, N-CH_2*CH_3*); ^{13}C NMR ($CDCl_3$): δ 166.1 (C=O); 156.3 (C-3); 155.8 (C-5); 149.5 (Me_2*C*=N); 133.6, 129.2, 129.0, 128.3 (Ar); 42.2 (N-*CH_2*CH_3); 38.1 (CH_2-1′); 15.1 (N-CH_2*CH_3*); 11.9 (C_5-*Me*). Anal. cacld for $C_{14}H_{17}N_5O$ (271.3): C, 61.98; H, 6.32; N; 25.81. Found: C, 61.78; H, 6.23; N, 25.68. MS: *m/z* (EI) 271 $(M)^+$.

4.2.4. (1-Ethyl-5-methyl-1H-[1,2,4]triazol-3-yl)-acetic acid furan-2-ylmethylene-hydrazide ***(11)***

From **7a** (0.92 g). Yield: 0.91 g, 70%, m.p. 137–138 °C. ^{1}H NMR ($CDCl_3$, 30 °C, two isomers): δ 11.52 (s, 1/2H, NH); 11.36 (s, 1/2H, NH); 8.11 (s, 1/2H, =C*H*furan); 7.81, 7.79 (2 × s, 1H, furan-H-3); 6.84, 6.88 (2 × d, 1H, furan-H-4); 6.61 (br s, 1H, furan-H-5); 7.90 (s, 1/2H, =C*H*furan); 4.05 (2 × q, 2H, *J* = 7.1 Hz, N-*CH_2*CH_3); 3.93 (s, 1/2H, CH_2-1′); 3.50 (s, 1/2H, CH_2-1′); 2.38, 2.35 (2 × s, 3H, C_5-Me); 1.29 (2 × t, 3H, N-CH_2*CH_3*); ^{1}H NMR ($CDCl_3$, 120 °C): δ 10.93 (s, 1H, NH); 8.06 (s, 1H, =C*H*furan); 7.68 (s, 1H, furan-H-3); 6.77 (d, 1H, *J* = 3.1 Hz, furan-H-4); 6.54 (dd, 1H, *J* = 2.0 Hz, 3.1 Hz, furan-H-5); 4.02 (q, 2H, *J* = 7.4 Hz, N-*CH_2*CH_3); 4.00 (s, 1H, CH_2-1′); 2.47 (s, 3H, C_5-Me); 1.32 (t, 3H, N-CH_2*CH_3*); ^{13}C NMR ($CDCl_3$, 30 °C, two isomers): δ 170.2, 164.4 (C=O); 156.3, 156.2 (C-3); 151.6, 151.4 (C-5); 149.3, 149.1 (C=N); 144.7, 145.0, 136.3, 133.0 (C-3, C-4, furan); 113.3, 112.8 (C-3, furan); 112.0, 111.9 (C-5, furan); 42.3, 42.2 (N-*CH_2*CH_3); 34.7, 31.8 (CH_2-1′); 14.7 (N-CH_2*CH_3*); 11.1 (C_5-*Me*); ^{13}C NMR ($CDCl_3$, 120 °C): δ 156.5 (C-3); 151.6 (C-5); 149.9 (C=N); 144.6, 145.5(furan); 42.3, 42.2 (N-*CH_2*CH_3); 34.7, 31.8 (CH_2-1′); 14.7 (N-CH_2*CH_3*); 11.1 (C_5-*Me*). Anal. cacld for $C_{12}H_{15}N_5O_2$ (261.3): C, 55.16; H, 5.79; N; 26.80. Found: C, 54.95; H, 5.68; N, 26.61. MS: *m/z* (EI) 261 $(M)^+$.

4.2.5. (1-Ethyl-5-methyl-1H-[1,2,4]triazol-3-yl)-acetic acid cyclopentylidin-2-ylmethylene hydrazide ***(12)***

From **7a** (0.92 g). Yield: 1.03 g, 83%, m.p. 147–159 °C. ^{1}H NMR ($CDCl_3$, 30 °C, two isomers)): δ 10.13 (s, 1/2H, NH); 10.01 (s, 1/2H, NH); 4.03, 3.98 (2 × q, 2H, *J* = 7.1 Hz, N-*CH_2*CH_3); 3.77 (s, 1/2H, CH_2-1′); 3.52 (s, 1/2H, CH_2-1′); 2.34, 2.32 (2 × s, 3H, C_5-Me); 2.30–2.24 (m, 2H, H-2, H-5-cyclopentane); 1.29 (2 × t, 3H, N-CH_2*CH_3*); ^{13}C NMR ($CDCl_3$, 30 °C, two isomers): δ 170.4, 166.1 (C=O); 164.1, 162.4 (C-1-cyclopentane); 156.8 (C-3); 151.4, 151.4 (C-5); 42.5, 42.4 (N-*CH_2*CH_3); 34.3, 33.1 (CH_2-1′); 32.8, 32.2, 28.3, 28.2 (C-2, C-5-cyclopentane); 24.6, 24.5, 24.4, 24.3 (C-3, C-4-cyclopentane; 14.8 (N-CH_2*CH_3*); 11.2 (C_5-*Me*). Anal. calcd for $C_{12}H_{19}N_5O$ (249.3): C, 57.81; H, 7.68; N; 28.09. Found: C, 57.60; H, 7.59; N, 27.89. MS: *m/z* (EI) 249 $(M)^+$.

*4.2.6. (6,7,8,9-Tetrahydro-5H-[1,2,4]-triazolo[1,5,-a]azepine-2-yl)-acetic acid **(13)***

A solution of **7c** (0.40 g, 1.91 mmol) was stirred with 1.0 M NaOH (10 ml) at 23 °C for 24 h. The solution was neutralized with 1.0 M HCl to pH 6.0. The separated solid was filtered, washed with a little EtOH then dried to give **13** (0.23 g, 62%); m.p. 75–76 °C. ^{1}H-NMR ($CDCl_3$): δ 4.46 (br s, 1H,CO_2H); 4.07 (br s, 2H, CH_2-5′); 3.29 (br s, 2H, CH_2-1); 2.76 (m, 2H, CH_2-9′); 1.74 (m, 2H, CH_2-7′); 1.64 (m, 2H, CH_2-6′); 1.53 (m, 2H, CH_2-6′); ^{13}C-NMR ($CDCl_3$): 176.3 (CO_2H); 158.4 (C-2′); 157.5 (C-10′); 51.5 (C-5′); 37.5 (CH_2-7′); 30.2 (CH_2-6′); 27.4 (CH_2-9′); 26.9 (C-1); 24.8 (CH_2-8′). Anal. cacld for $C_9H_{13}N_3O_2$ (195.2): C, 55.37; H, 6.71; N; 21.52. Found: C, 55.18; H, 6.68; N, 21.04. MS: *m/z* (FAB) 196 $(MH)^+$.

*4.2.7. 1,5-Dimethyl-3-(5-mercapto-4-N-phenyl-1H-[1,2,4]-triazol-3-ylmethylene)-1H-[1,2,4] triazole **(14)***

To a suspension of **7a** (0.50 g, 2.73 mmol), phenyl isothiocyanate (0.29 g, 2.39 mmol) was added and the mixture was heated under reflux for 4 h after cooling, the thiosemicarbazide solid was filtered, washed with EtOH, dried and used for the next step. The solid was dissolved in 5% NaOH solution (25 ml) and boiled for 3 h. After cooling, the solution was treated with charcoal, filtered and acidified with dil HCl to give **14** (0.46 g, 67%) as a solid, m.p. 246–247 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.8 (s, 1H, NH); 7.43 (m, 3H, ArH); 7.27 (m, 2H, ArH); 3.81 (s, 2H, CH_2-1′); 3.58 (s, 3H, NMe); 2.24 (s, 3H, C_5-Me); ^{13}C NMR (DMSO-d_6): δ 168.0 (C=S); 155.7 (C-3); 152.7 (C-5); 149.5 (C-3”); 133.6, 129.3, 129.1, 128.3 (C-Ar); 34.7 (NMe); 25.5 (C-1′); 11.2 (C_5-*Me*). Anal. cacld for $C_{13}H_{14}N_6S$ (286.4): C, 54.53; H, 4.93; N; 29.35. Found: C, 54.34; H, 4.86; N, 29.169. MS: *m/z* (EI) 286$(M)^+$.

*4.2.8. 1,5-Dimethyl-3-(5-ethylsulfanyl-4-N-phenyl-1H-[1,2,4]-triazol-3-ylmethylene)-1H-[1,2,4] triazole **(15)***

To a solution of **14** (0.50 g, 1.74 mmol) in THF (3 ml) cooled at 0 °C was added a solution of 1 N NaOH (1.60 ml) under nitrogen atmosphere. After the mixture stirred for 15 min, ethyl iodide (140 μl) was added. After stirring at 0 °C for additional 1 h, solvents were evaporated to dryness furnished a pale-yellow solid. Recrystallization from CH_3CN-H_2O gave **15** (0.41 g, 75%) as a crystals, m.p. 261–265 °C. ^{1}H NMR (DMSO-d_6): δ 13.8 (s, 1H, NH); 7.39 (m, 3H, ArH); 7.24 (m, 2H, ArH); 3.74 (s, 2H, CH_2-1′); 3.55 (s, 3H, NMe); 2.23 (s, 3H, C_5-Me). 3.50 (q, 2H, J = 7.0 Hz, S*CH*$_2$CH$_3$); 1.31 (t, 3H, SCH$_2$*CH*$_3$). Anal. cacld for $C_{15}H_{18}N_6S$ (314.4): C, 57.30; H, 5.77; N; 26.73. Found: C, 57.01; H, 5.59; N, 26.54. MS: *m/z* (FAB) 337$(MNa)^+$.

*4.2.9. 4-(4-Methoxyphenyl)-1H-[1,2,4]-triazolo[1,5-a]-pyridin-2-ylmethylene)-[1,2,4]-triazole-3-thione **(16)***

From **7d** (0.65 g, 3.84 mmol) and 4-methoxyphenyl-isocyanate (0.50 g, 3.33 mmol). Yield: 0.82 g (62%); m.p. 267–269 °C. ^{1}H NMR (DMSO-d_6): δ 12.98 (s, 1H, NH); 4.19 (t, 2H, J = 6.0 Hz, CH_2-5″); 3.87 (s, 2H, CH_2-1′); 3.85 (s, 3H, OMe); 2.87 (t, 2H, J = 6.0 Hz, CH_2-8″); 2.21-1.59 (m, 4H, CH_2-6″, CH_2-7″); ^{13}C NMR (δ, DMSO-d_6): 167.0 (C=S); 159.2 (Ar*C*-OMe); 156.9 (C-2″); 152.9 (C-9″); 149.3 (C-5); 146.2, 129.6, 114.6 (C-Ar); 54.9 (Ar-OMe); 46.1 (C-5″); 25.8 (C-1′); 24.5 (C-6″); 23.7 (C-7″); 20.7 (C-8″). Anal. cacld for $C_{16}H_{18}N_6OS$ (342.4): C, 56.12; H, 5.30; N; 24.54. Found: C, 55.90; H, 5.22; N, 24.35. MS: *m/z* (EI) 342 $(M)^+$.

*4.2.10. 5-(6,7,8,9-Tetrahydro-5H-[1,2,4]-triazolo[1,5,-a]-azepine-2-ylmethylene)-[1,3,4] oxadiazole-2-thione **(17)***

To a solution of **7c** (0.50 g, 2.39 mmol) in EtOH (10 ml) containing KOH (200 mg, 3.58 mmol), CS_2 (2 ml) were added. The reaction mixture was heated under reflux for 8 h, with stirring, then concentrated, cooled, and acidified with diluted HCl. The separated product was filtered, washed with water, and recrystallized from EtOH to give **17** (0.50 g, 83%), m.p. 251–252 °C. ^{1}H NMR (DMSO-d_6): δ 7.98 (br s, 1H, NH); 4.52 (m, 2H, CH_2-5″); 4.32 (s, 2H, CH_2-1′); 3.00 (br s, 2H, CH_2-9″); 1.84 (br s, 2H, CH_2-7″); 1.77 (br s, 2H, CH_2-6″); 1.68 (br s, 2H, CH_2-8″); ^{13}C-NMR ($CDCl_3$): 178.0 (C=S); 158.9 (C-5-oxathiadiazole); 159.5 (C-2″); 157.2 (C-10″); 51.4 (C-5″); 38.7 (CH_2-7″); 29.1 (CH_2-6″); 26.8 (CH_2-9″); 25.6 (C-1); 24.4 (CH_2-8″). Anal. cacld for $C_{10}H_{13}N_5OS$ (251.3): C, 47.79; H, 5.21; N; 27.87. Found: C, 47.54; H, 5.13; N, 27.68. MS: *m/z* (EI) 251 $(M)^+$.

*4.2.11. 2-(6,7,8,9-Tetrahydro-5H-[1,2,4]-triazolo[1,5,-a]-azepine-2-ylmethylene)-5-ethylmercapto-1,3,4-oxadiazole **(18)***

This compound was prepared from **17** (0.50 g, 1.99 mmol), by the procedure of preparation of **15** to furnish a pale-yellow solid. Recrystallization from CH_3CN-H_2O gave **18** (0.46 g, 82%) as a crystals, m.p. 276–279 °C. ^{1}H NMR (DMSO-d_6): δ 4.21 (m, 4H, CH_2-5″; CH_2-1′); 3.52 (q, 2H, J = 7.1 Hz, S*CH*$_2$CH$_3$); 2.83 (m, 2H, CH_2-9″); 1.81 (m, 2H, CH_2-7″); 1.75 (m, 2H, CH_2-6″); 1.65 (br s, 2H, CH_2-8″); 1.32 (t, 3H, SCH$_2$*CH*$_3$). Anal. cacld for $C_{12}H_{17}N_5OS$ (279.4): C, 51.59; H, 6.13; N; 25.07. Found: C, 51.38; H, 6.04; N, 24.88. MS: *m/z* (EI) 279$(M)^+$.

*4.2.12. 5-(1-Ethyl-5-methyl-1H-[1,2,4]-triazol-3-ylmethylene)-3H-[1,3,4]oxadiazole-2-thione **(19)***

From **7a** (0.55 g, 3.00 mmol) in the manner described for **14**. Yield: 0.52 g (77%); m.p. 239–243 °C; ^{1}H NMR ($CDCl_3$): δ 8.10 (br s, 1H, NH); 4.29 (s, 2H, CH_2-1′) 3.94 (q, 2H, J = 7.3 Hz, N-*CH*$_2$CH$_3$); 2.57 (s, 3H, C_5-Me); 1.11 (s, 3H, N-CH$_2$*CH*$_3$); ^{13}C NMR (δ, $CDCl_3$): 176.9 (C=S); 158.5 (C-5-oxathiadiazole); 41.8 (N-*CH*$_2$CH$_3$); 25.4 (CH_2-1′); 15.0 (N-CH$_2$*CH*$_3$); 11.4 (C_5-*Me*). Anal. cacld for $C_8H_{11}N_5OS$ (225.3): C, 42.65; H,4.92; N, 31.09. Found: C,42.42; H,4.93; N, 30.93. MS: *m/z* (EI) = 225 $(M)^+$.

*4.2.13. 5-(1,5-Diethyl-1H-[1,2,4]-triazol-3-ylmethylene)-3H-[1,3,4]oxadiazole-2-thione **(20)***

From **7b** (0.60 g, 3.04 mmol) in the manner described for **14**. Yield: 0.58 g (79%); m.p. 191–193 °C; ^{1}H NMR

($CDCl_3$): δ 7.86 (s, 1H, NH); 4.20 (s, 2H, CH_2-1′); 3.90 (q, 2H, J = 7.5 Hz, N-CH_2CH_3); 2.54 (s, 3H, C_5-Me); 1.11 (s, 3H, N-CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 177.0 (C=S); 159.1 (C-5-oxathiadiazole); 42.5 (N-CH_2CH_3); 14.8 (C_5-CH_2CH_3); 11.6 (N-CH_2CH_3); 11.5 (C_5-CH_2CH_3). Anal. cacld for $C_9H_{13}N_5OS$: C, H, N. Found: C, H, N. MS: *m/z* (EI) = 239 $(M)^+$.

4.2.14. 5-(1,5-Diethyl-1H-[1,2,4]-triazol-3-ylmethylene)-[1,3,4]oxadiazol-2ylamine ***(21)***

A solution of **7b** (0.30 g, 1.52 mmol) and CNBr (0.50 g, 4.70 mmol) in EtOH (25 ml) was heated at 70 °C for 3 h. After cooling, the mixture was neutralized with Na_2CO_3 and poured into crushed ice. The solid product was isolated and recrystallized from DMF to give **21** as a brown crystals (0.26 g, 76%); m.p. 139–140 °C. ^{1}H NMR ($CDCl_3$): δ 6.79 (br s, 2H, NH_2); 3.97 (s, 2H, CH_2-1′); 3.87 (q, 2H, J = 7.0 Hz, N-CH_2CH_3); 2.60 (q, 2H, C_5-CH_2CH_3); 1.11 (t, 3H, N-CH_2CH_3); 1.03 (t, 3H, C_5-CH_2CH_3). Anal. cacld for $C_9H_{14}N_6O$ (222.3): C, 48.64; H, 6.35; N, 37.81. Found: C, 48.36; H, 6.21; N, 37.60. MS: *m/z* (FAB) = 223 $(MH)^+$; 245 (MNa^+).

4.2.15. 1-(6,7,8,9-Tetrahydro-5H-[1,2,4]-triazolo[1,5,-a]-azepine-2-yl)benzyl]indole ***(25)***

This compound was prepared according to the reported procedure [39], from reaction of 1-(6,7,8,9-tetrahydro-5*H*-[1,2,4],2,4-triazolo[1,5,-*a*]azepine-2-yl)-4-benzyl chloride **23** (0.52 g, 2.00 mmol) with indole **24** (1.50 mmol) in the presence of NaH (2.00 mmol, 60% in oil). Yield: 0.52 g, 76%; m.p. 222–225 °C. All the physical data are identical to those of the authentic sample prepared previously.

4.2.16. 1-(1-Ethyl-5-methyl-1H-[1,2,4]-triazol-3-yl)benzyl]-indole ***(27)***

This compound was prepared according to the reported procedure [39], from coupling of 1-ethyl-5-methyl-3-[4-chlorobenzyl-1-yl]-1*H*-1,2,4-triazole **26** (0.49 g, 2.00 mmol) with indole **24** (1.50 mmol) in the presence of NaH (2.00 mmol, 60% in oil). Yield: 0.52 g, 82%; m.p. 232–235 °C. All the physical data are identical to those of the authentic sample prepared previously.

5. Pharmacological experiments

5.1. Primary cancer assay

Compounds were evaluated in an in vitro model in the 3-cell line panel, one dose assay. Each cell line [MCF7 (breast)], NCI-H4460 (lung) and SF-268 (CNS) was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration (10^{-4} M) and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as % of growth of the treated cells when compared to untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range [48,49].

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