Full Paper

Stereoselective Synthesis and Antiviral Activity of (1*E*,2*Z*,3*E*)-1-(Piperidin-1-yl)-1-(arylhydrazono)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-4-(aryl[1])but-3-enes

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The reaction of benzoyl hydrazine **1a** or benzothiazole-2-carbohydrazide **1b** with 2-oxo-*N*-arylpropanehydrazonoyl chlorides **2a–d** yielded (1*Z*,2*E*)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-*N*-(aryl)-propanehydrazonoyl chlorides **3a–e**. The reaction of **3a–c** with sodium benzenesulphinate furnished sulphones **5a–c** while the reaction of **5d**, **e** with hydroxyl amine afforded hydroxomoyl derivatives **6a**, **b**. The one-pot sterioselective reaction of *N*-(aryl)propanehydrazonoyl chlorides **3** with certain aromatic aldehydes in the presence of piperidine resulted in the formation of (1*E*,2*Z*,3*E*)-1-(piperidin-1-yl)-1-(arylhydrazono)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-4-(aryl[1])-but-3-enes **7a–g**. X-ray analysis of piperidinyl amidrazone **7g** showed a conversion of its geometrical structure with respect to that of compound **3** and confirmed the stereoselectivity of the latter reaction. The piperidinyl amidrazones **7a–g** possessed a significant antiviral activity against herpes simplex viruses (HSV-1). Compound **7d** reduced the number of viral plaques of herpes simplex type-1 (HSV-1) by 67%, with respect to the effect of reference drug Aphidicolin.

Keywords: Antiviral activity / Benzothiazole / HSV-1 / Hydrazones / X-ray crystallography

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Introduction

Viral infections are considered one of the principle threats to human life and health worldwide. The viral diseases caused by herpes simplex viruses, HSV-1 and HSV-2, were categorized into several distinct disorders based on the site of infection. HSV infection may affect the face (orofacial herpes), genitalia (genital herpes), hand (herpes whitlow), eye (herpes keratitis), or the brain (herpes encephalitis) [1–4]. The common opportunistic ocular infections, corneal epithelial and stromal keratitis, are caused by herpes simplex virus, HSV-1; they are a leading cause of blindness [5]. Moreover, AIDS patients are prone to severe complications from HSV infections. HSV-1 appeared to be particularly damaging to the nervous system, and it increases the risk of developing Alzheimer's disease [6–8].

Hydrazones have been reported as useful antiviral agents. Some acetylhydrazones revealed a remarkable antiviral activity against HSV-1 [9], whereas arylhydrazones inhibited the replication of HIV-1 [10]. Furthermore, an excellent antiviral activity has been demonstrated by several heteroarylhydrazones versus influenza A_2, A_3, and semliki forest viruses [11]. Additionally, 2-substituted benzothiazoles were intensively studied as antiviral agents. For example, the *in-vivo* antiviral activity of 2-aminobenzothiazole has a potency quite comparable to that of the antiviral drug Amantadine against influenza A_2 strains [12]. A series of 2-substituted benzothiazoles have been reported for treating HIV infection and AIDS [13], also some amidino benzothiazoles have been described as potent anti-HIV agents [14]. Moreover, fused benzothiazoles exerted antiviral activity versus herpes simplex virus HSV-1 [15].

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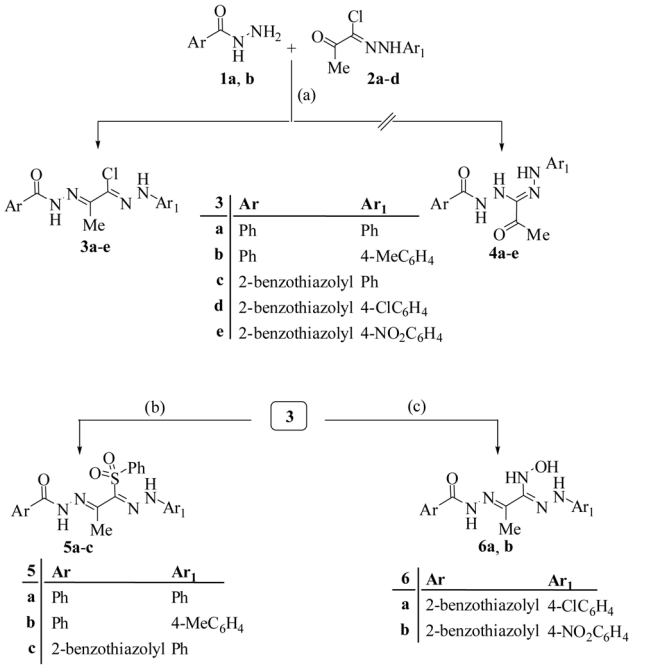


Reagents and conditions: (a) EtOH, reflux 9 h, 70–79%; (b) $PhSO_2Na \cdot 2\ H_2O$, reflux 18 h, 52–58%; (c) $NH_2OH \cdot HCl$, K_2CO_3, EtOH, reflux 45 min, 50–56%.

Scheme 1. Synthesis of compounds **3a–e**, **5a–c** and **6a–b**.

Considering the above-mentioned facts and in the light of our ongoing research of developing new molecules with potent antiviral activity against herpes simplex virus HSV-1 [16–20], particularly, with respect to its risk in AIDS and Alzheimer patients, we synthesized a series of benzoyl/benzothiazoloyl hydrazones bearing a sulfone or piperidine moiety for their antiviral activity against herpes simplex viruses HSV-1. The cytotoxicity of the newly synthesized compounds was estimated.

Results and discussion

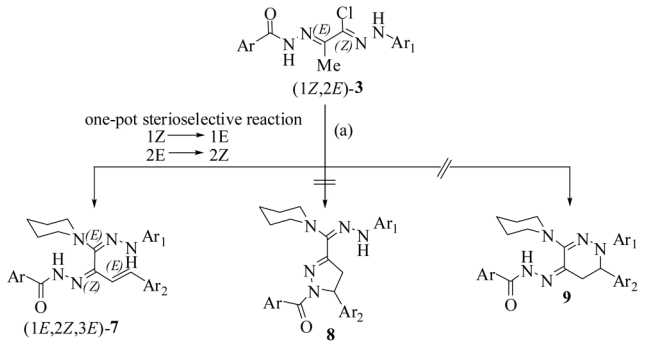
Chemistry

Our previous work on the chemistry of bi-hydrazones [21] as part of a program, directed us towards the synthesis of biologically active heterocycles [21–27]. In the present study, we aim to report the synthesis of (1*Z*,2*E*)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-*N*-(aryl)propanehydrazonoyl chloride **3a–e** (Scheme 1). Thus, the reaction of benzoyl hydrazine **1a** or benzothiazole-2-carbohydrazide **1b** with 2-oxo-*N*-arylpropanehydrazonoyl chlorides **2a–d** in refluxing ethanol afforded, in each case, a single product. IR spectra of the latter products exhibited a carbonyl absorption band in the region 1683–1652 cm^{-1} in addition to the absorption bands of two NH functions in the region ν 3455–3182 cm^{-1}. Their ^{1}H-NMR showed two D_2O exchangeable signals of two NH groups in the regions δ: 9.56–10.04 and δ: 10.87–11.16 ppm in addition to the singlet signal of the methyl group in the region δ: 2.31–2.34 ppm. The analyses of the latter products confirmed the assigned structure **3** (Scheme 1). Recently, we reported the X-ray diffraction for the benzofuran analogue of structure **3**. It confirmed that the stereochemistry of such a class of bi-hydrazones is the (1*Z*,2*E*)-configuration [21].

Arylsulphones are a promising class of antiviral agents. Arylsulphones of indole [28], thiophene [29], and pyrrole [30] have been shown to display interesting antiviral activity. Some derivatives of **3** have been selected for the synthesis of new bi-hydrazones bearing a phenylsulphone moiety. Thus, reaction of **3a–c** with sodium benzenesulphinate afforded the corresponding sulphones **5a–c**, respectively (Scheme 1). The structure of the latter sulphones was assigned by their IR, ^{1}H-NMR, and MS spectra.

Moreover, the reaction of **3d** or **3e** with hydroxylamine hydrochloride, in the presence of potassium carbonate, yielded the corresponding *N*-hydroxy-2-(2-(benzothiazol-2-carbonyl)hydrazono)-*N*′-(4-aryl)propanehydrazonamide **6a**, **b**, respectively (Scheme 1). The structure of **6a**, **b** was confirmed on the basis of their spectroscopic data. For example, the IR spectrum of **6a** showed absorption bands in the region 3410–3120 cm^{-1} of three NH groups and an OH function, and the band of the carbonyl group at 1680 cm^{-1}. Its ^{1}H-NMR spectrum revealed four D_2O exchangeable singlet signals (three NH + OH), whereas its mass spectrum showed a peak corresponding to its molecular ion at *m*/*z*: 402 [M^+].

The piperidine motif has been reported as a key structural component of several successful antiviral drug candidates [31–40] where piperidine-based phenoxy [34], pyrazole [35], alkylamine [36], carboxamide [37], sulphone [38], alkene [39], or diketone [40] derivatives possessed a unique antiviral activity. From the above mentioned facts and in continuation of our interest in the synthesis of poly-functionally piperidines of expected biological importance [21, 24], we report here the utility of bi-hydrazones **3a–e** in a one-pot stereoselective synthesis of the title compounds. Thus, condensation of **3a–e** with certain aromatic aldehydes in refluxing ethanol in the presence of four molar ratios of piperidine afforded the reaction product which precipitated during reflux. The IR spectra of the latter products exhibited, in each case, a band in the region 1686–1639 cm^{-1} due to the carbonyl absorption; also, the absorption bands of two NH functions appeared in the region 4012–3118 cm^{-1}. The ^{1}H-NMR spectra of the latter products showed two signals in the regions δ: 8.89–9.52 and 10.47–10.88 ppm assigned to



7	Ar	Ar_1	Ar_2
a	Ph	Ph	Ph
b	Ph	Ph	4-$MeOC_6H_4$
c	Ph	4-MeC_6H_4	Ph
d	Ph	4-MeC_6H_4	2-theinyl
e	2-benzothiazolyl	Ph	Ph
f	2-benzothiazolyl	4-ClC_6H_4	Ph
g	2-benzothiazolyl	4-$NO_2C_6H_4$	Ph

Reagents and conditions: (a) Appropriate aldehyde, EtOH, piperidine (4 eq.), reflux 8 h, 64–75%.

Scheme 2. Synthesis of compounds **7a–g**.

the two NH groups. The ^{1}H-NMR spectra of the reaction products revealed that the olefinic protons were considerably shifted downfield appearing in the aromatic region. The spectroscopic data of the latter provide support for the suggested structure **7** (Scheme 2) and ruled out both of the expected cyclized products **8** and **9** (Scheme 2). However, X-ray analysis of amidrazone **7g** not only excluded the other possible products but also showed the structural geometry of **7g** as (1*E*,2*Z*,3*E*)-configuration (Figs. 1a and 1b). X-ray analysis of amidrazone **7g** showed a conversion of the configuration with respect to the geometry of **3** and it confirmed both the stereoselectivity of the latter reaction and the *E* configuration of the amidrazone skeleton (1*E* = 170.1°). The selected bond distances of **7g** and torsion angles of three essential double bonds 1*E*, 2*Z*, and 3*E* of (1*E*,2*Z*,3*E*)-**7g** are illustrated in Table 1.

In-vitro anti-herpes simplex-1 virus (HSV-1) and cytotoxicity assays

Compounds **5a–c**, **6a**, **b**, and **7a–g** were tested for their possible antiviral and cytotoxicity activity. The compounds were tested for antiviral activity against herpes simplex type 1 (HSV-1) grown on *Vero* African green monkey kidney cells. An improved plaque-reduction assay for antiviral activity was used to test our compounds. Plaque-reduction assays typically use a monolayer of cultured host cells which are allowed to bind virus, then, they are overlayed with a layer of medium thickened with agar or

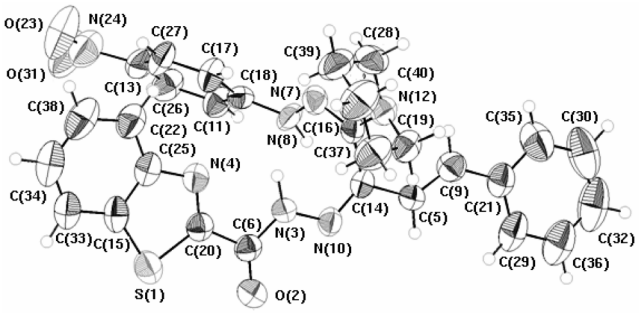
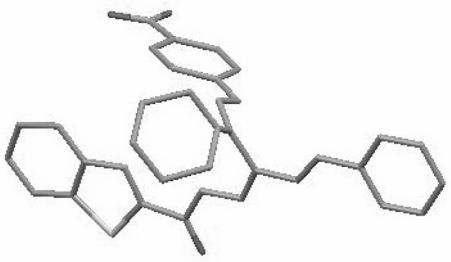


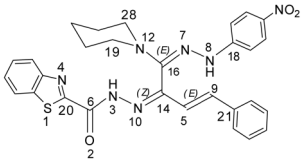
Figure 1a. X-ray structure of (1E,2*Z*,3*E*)-**7g**.



Hydrogen atoms are omitted for clarity.

Figure 1b. View of (1*E*,2*Z*,3*E*)-**7g**.

Table 1. Characteristic bond lengths (Å) and torsion angles (°) of (1*E*,2*Z*,3*E*)-**7g**.



bond length (Å)			
N8–C18	1.375 (4)[§]	N3–C6	1.349 (4)
N7–N8	1.389 (4)[&]	O2–C6	1.218 (4)
N7–C16	1.304 (4)[#]	C6–C20	1.505 (5)
N12–C16	1.358 (4)[†]	S1–C20	1.727 (4)
N12–C19	1.468 (4)	N4–C20	1.298 (4)
N12–C28	1.466 (5)	C5–C14	1.460 (4)
C14–C16	1.503 (5)[‡]	C5–C9	1.306 (4)
N10–C14	1.293 (4)	C9–C21	1.464 (5)
N3–N10	1.362 (3)		
torsion angles (°)			
(1*E*) N12–C16–N7–N8		170.1 (8)[**]	
(2*Z*) C16–C14–N10–N3		0.8 (5)[*]	
(3*E*) C21–C5–C9–C21		−179.7 (11)[**]	

The bond lengths of (1*E*,2*Z*,3*E*)-**7g** are well within the range that typically occurred in other amidrazone derivatives: § 1.391(2)–1.396(3) Å; & 1.342(4)–1.351(2) Å; # 1.286(3)–1.294(2) Å; † 1.373(3)–1.405(4) Å, and ‡ 1.491(4)–1.511(3) Å [21]. The torsion angles of (1*E*,2*Z*,3*E*)-**7g** are also well within the range that is typically reported: * *Z* = 0° → ± 30° and ** *E* = ± 150° → 180°.

Table 2. Results of *in vitro* anti-herpes simplex-1 virus (HSV-1) and cytotoxicity.

Compound	% Reduction[§]	MAC[&]	CD_{50}[#]
5a	None	–	0.02
5b	None	–	0.02
5c	None	–	0.04
6a	None	–	0.01
6b	None	–	0.02
7a	22	0.1	0.02
7b	29	0.1	0.03
7c	16	0.1	0.01
7d	67	0.1	0.02
7e	28	0.1	0.01
7f	62	0.1	0.02
7g	33	0.1	0.01
Aphidicolin[†]	100	0.005	0.2

§ Percent (%) reduction in the number of viral plaques; & minimum antiviral concentration (in mg/mL); # cytotoxicity (compound concentration caused 50% loss of the monolayer present around the viral plaques) (in mg/mL); † positive antiviral control; –: Not detected.

another thickener which makes plaque formation possible by preventing mixing due to currents in the medium. Samples to be tested for antiviral activity are either incorporated into the thickened layer or absorbed in a paper disc placed on the thickened layer. The thickened layer can cause several types of technical problems. In his assay, Shier reduced the assay size to fit the wells of 96-well microliter trays. Also he used serial dilutions of samples in parallel wells in a tray allowing an estimation of end-point concentrations for antiviral agents. Shier's modifications reduced the sample size, the cost, and eliminated the potential for interference by thickening agents. At the same time, this assay retains the ability to estimate the cytotoxicity which is reflected as loss of the cell monolayer in which the plaques are normally formed [41–43].

The modified assay was used to examine compounds **5a–c**, **6a**, **b**, and **7a–g**. Aphidicolin was used as a positive control. All compounds exhibit cytotoxicity and no selective antiviral activity.

Sulphones **5a–c** and hydroxomoyles **6a**, **b** showed no antiviral activity but piperidinyl amidrazones **7a–g** showed a weak to moderate activity in reducing the number of plaques at the same concentration (0.1 mg/mL). These results imply that the piperidine moiety attached to the bi-hydrazone backbone is an essential pharmacophoric site. Compound **7d** was the best among the tested compounds; it reduced the number of viral plaques of herpes simplex type-1 (HSV-1) by 67%, with respect to the reference drug aphidicolin. Replacing the thienylidine moiety in **7d** by a benzylidine group as in **7c** decreased the antiviral activity. Similarly, the derivative **7b** that contained the 4-methoxybenzylidine group, showed slightly higher inhibition of HSV-1 (29%) than that of the parent amidrazone **7a** with the benzylidine group (23%). On the other hand, compound **7f** with a 4-chlorophenylhydrazone group showed 62% reduction of the HSV-1 plaques, and, replacing this group by 4-nitrophenylhydrazone as in **7g**, lowered the antiviral activity to 33%. Taken together, the results of **7a–g** provide evidence that the structural features of the arylidine moieties and changes in the aryl group of the amidrazone skeleton significantly affects their anti-herpes simplex-1 virus (HSV-1) activity.

In conclusion, we described a facile synthesis of polyfunctionally (1*Z*,2*E*)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-*N*-(aryl)propanehydrazonoyl chloride bearing two activated centers which were used as electrophilic substitution substrates or *C*-nucleophiles. Some of the new piperidinyl amidrazones showed a significant antiviral activity against herpes simplex type-1 (HSV-1).

Experimental

Chemistry

Melting points were measured with a Gallenkamp apparatus (Weiss-Gallenkamp, London, UK). IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer (Shimadzu, Tokyo, Japan). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ^{1}H-NMR spectra were run at 300 MHz in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard. All coupling constant (*J*) values are given in Hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; D_2O exch., exchanged with D_2O; all the NH exchanged with D_2O. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University and are within ± 0.4% of the theoretical value. X-ray crystallography was carried out on a Kappa CCD Enraf Nonius FR 590 diffractometer, National Research Center, Dokki, Cairo, Egypt. Benzothiazole-2-carbohydrazide **1b** [44] and 2-oxo-*N*-arylpropanehydrazonoyl chlorides **2a–d** [45] were prepared according to the literature procedures. Benzoyl hydrazine **1a** was used as obtained commercially. The reactions were monitored and the purity of the products was checked by TLC plates (0.25 mm silica gel, aluminum sheets 60 F_{254}, Merck, Germany). The spots were visualized using 254 nm UV lamp.

*General procedure for the synthesis of (1Z,2E)-2-(benzoyl/benzothiazol-2-oyl)-N-arylpropanehydrazonoyl chlorides **3a–e***

A mixture of benzoyl hydrazine **1a** or benzothiazole-2-carbohydrazide **1b** (10 mmol) and 2-oxo-*N*-arylpropanehydrazonoyl chloride **2a–d** (10 mmol) in absolute ethanol (50 mL) was

refluxed for 9 h. Then left to cool, the formed solid was filtered off, washed with ethanol, and recrystallized from EtOH/DMF to afford the corresponding hydrazonoyl chlorides **3a–e**, respectively.

(1Z,2E)-2-(Benzoylhydrazono)-N-phenylpropanehydrazonoyl chloride ***3a***

Pale yellow powder, yield (78%); m. p.: 198–200°C; IR (KBr) ν_{max} cm^{-1}: 3455, 3303 (2 NH), 1683 (C=O), 1593 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 7.05–7.98 (m, 10H, ArH), 9.74 (s, D_2O exch., 1H, NH), 10.94 (s, D_2O exch., 1H, NH); MS *m/z* (%): 316 [M^+ + 2] (3.1), 315 [M^+ + 1] (2.2), 314 [M^+] (8.9), 77 (100). Anal. calcd. for $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80. Found: C, 60.82; H, 4.88; N, 17.93.

(1Z,2E)-2-(Benzoylhydrazono)-N-(4-tolyl)propanehydrazonoyl chloride ***3b***

Yellow powder, yield (74%); m. p.: 203–205°C; IR (KBr) ν_{max} cm^{-1}: 3390, 3272 (2 NH), 1668 (C=O), 1590 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.10 (d, *J* = 7.5 Hz, 2H, ArH), 7.23 (d, *J* = 7.5 Hz, 2H, ArH), 7.35–7.93 (m, 5H, ArH), 10.04 (s, D_2O exch., 1H, NH), 10.87 (s, D_2O exch., 1H, NH); MS *m/z* (%): 330 [M^+ + 2] (4.3), 329 [M^+ + 1] (3.0), 328 [M^+] (14.1), 77 (100). Anal. calcd. for $C_{17}H_{17}ClN_4O$: C, 62.10; H, 5.21; N, 17.04. Found: C, 61.88; H, 5.35; N, 16.90.

(1Z,2E)-2-{[(Benzothiazol-2-yl)carbonyl]hydrazono}-N-phenylpropanehydrazonoyl chloride ***3c***

Yellow powder, yield (75%); m. p.: 216–218°C; IR (KBr) ν_{max} cm^{-1}: 3409, 3294 (2 NH), 1680 (C=O), 1595 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 7.09–7.89 (m, 9H, ArH), 9.84 (s, D_2O exch., 1H, NH), 10.93 (s, D_2O exch., 1H, NH); MS *m/z* (%): 373 [M^+ + 2] (2.1), 372 [M^+ + 1] (1.5), 371 [M^+] (6.5), 135 (100). Anal. calcd. for $C_{17}H_{14}ClN_5OS$: C, 54.91; H, 3.79; N, 18.83; S, 8.62. Found: C, 55.07; H, 3.67; N, 18.87; S, 8.55.

(1Z,2E)-2-{[(Benzothiazol-2-yl)carbonyl]hydrazono}-N-(4-chlorophenyl)propanehydrazonoyl chloride ***3d***

Yellow powder, yield (79%); m. p.: 237–239°C; IR (KBr) ν_{max} cm^{-1}: 3316, 3182 (2 NH), 1652 (C=O), 1588 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 7.12–7.98 (m, 8H, ArH), 10.03 (s, D_2O exch., 1H, NH), 11.16 (s, D_2O exch., 1H, NH); MS *m/z* (%): 409 [M^+ + 3] (6.7), 408 [M^+ + 2] (17.4), 407 [M^+ + 1] (56.8), 406 [M^+] (16.1), 135 (100). Anal. calcd. for $C_{17}H_{13}Cl_2N_5OS$: C, 50.26; H, 3.23; N, 17.24; S, 7.89. Found: C, 50.35; H, 3.17; N, 17.19; S, 7.83.

(1Z,2E)-2-{[(Benzothiazol-2-yl)carbonyl]hydrazono}-N-(4-nitrophenyl)propanehydrazonoyl chloride ***3e***

Yellow powder, yield (70%); m. p.: 276–278°C; IR (KBr) ν_{max} cm^{-1}: 3350, 3208 (2 NH), 1664 (C=O), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 7.11–8.02 (m, 8H, ArH), 9.56 (s, D_2O exch., 1H, NH), 10.83 (s, D_2O exch., 1H, NH); MS *m/z* (%): 418 [M^+ + 2] (4.8), 417 [M^+ + 1] (2.5), 416 [M^+] (12.8), 135 (100). Anal. calcd. for $C_{17}H_{13}ClN_6O_3S$: C, 48.98; H, 3.14; N, 20.16; S, 7.69. Found: C, 49.13; H, 3.18; N, 20.11; S, 7.74.

Synthesis of 1-phenylsulfonyl-1-arylhydrazono-2-(benzoyl/benzothiazol-2-oyl)propane ***5a–c***

To a solution of the appropriate propanehydrazonoyl chloride **3a–c** (1 mmol) in absolute ethanol (50 mL), sodium benzenesulphinate dihydrate (0.4 g, 2 mmol) was added. The mixture was refluxed for 18 h, then left to cool. The reaction mixture was poured into cold water and the solid product filtered off, washed with water, dried, and finally recrystallized from EtOH/DMF to afford the corresponding sulphones **5a–c**.

1-Phenylsulfonyl-1-phenylhydrazono-2-(benzoylhydrazono)propane ***5a***

Yellow powder, yield (58%); m. p.: 215–217°C; IR (KBr) ν_{max} cm^{-1}: 3300–3180 (2 NH), 1669 (C=O), 1599 (C=N), 1292, 1135 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 6.91–7.97 (m, 15H, ArH), 11.66 (s, D_2O exch., 1H, NH), 14.52 (s, D_2O exch., 1H, NH); MS *m/z* (%): 420 [M^+] (4.1), 77 (100). Anal. calcd. for $C_{22}H_{20}N_4O_3S$: C, 62.84; H, 4.79; N, 13.32; S, 7.63. Found: C, 63.02; H, 4.75; N, 13.32; S, 7.51.

1-Phenylsulfonyl-1-(4-tolylhydrazono)-2-(benzoylhydrazono)propane ***5b***

Yellow powder, yield (55%); m. p.: 245–247°C; IR (KBr) ν_{max} cm^{-1}: 3300–3175 (2 NH), 1641 (C=O), 1582 (C=N), 1293, 1138 (SO_2); ^{1}H-NMR (DMSO-d_6) *d*: 2.25 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 6.84 (d, *J* = 7.5 Hz, 2H, ArH), 7.10 (d, *J* = 7.5 Hz, 2H, ArH), 7.55–7.95 (m, 10H, ArH), 11.63 (s, D_2O exch., 1H, NH), 14.52 (s, D_2O exch., 1H, NH); MS *m/z* (%): 434 [M^+] (11.5), 77 (100). Anal. calcd. for $C_{23}H_{22}N_4O_3S$: C, 63.58; H, 5.10; N, 12.89; S, 7.38. Found: C, 63.44; H, 4.97; N, 12.96; S, 7.35.

1-Phenylsulfonyl-1-phenylhydrazono-2-{[(benzothiazol-2-yl)carbonyl]hydrazono}propane ***5c***

Yellow powder, yield (52%); m. p.: 247–249°C; IR (KBr) ν_{max} cm^{-1}: 3350–3180 (2 NH), 1655 (C=O), 1598 (C=N), 1292, 1135 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 6.67–8.18 (m, 14H, ArH), 11.65 (s, D_2O exch., 1H, NH), 14.50 (s, D_2O exch., 1H, NH); MS *m/z* (%): 477 [M^+] (6.2), 77 (100). Anal. calcd. for $C_{23}H_{19}N_5O_3S_2$: C, 57.85; H, 4.01; N, 14.66; S, 13.43. Found: C, 57.66; H, 4.13; N, 14.65; S, 13.28.

Synthesis of N-hydroxy-2-(2-(benzothiazol-2-oyl) hydrazono)-N′-(4-aryl)propanehydrazonamide ***6a, b***

A mixture of **3d** or **3e** (1 mmol) of hydroxylamine hydrochloride (0.11 g, 1.5 mmol) and anhydrous potassium carbonate (0.21 g, 1.5 mmol) in ethanol (50 mL) was refluxed for 45 min, then left to cool. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried, and finally recrystallized from EtOH/DMF to afford **6a** and **6b**, respectively.

N-Hydroxy-2-(2-(benzothiazol-2-oyl)hydrazono)-N′-(4-chlorophenyl)propanehydrazonamide ***6a***

Yellow powder, yield (50%); m. p.: >300°C; IR (KBr) ν_{max} cm^{-1}: 3410–3120 (3 NH + OH), 1680 (C=O), 1589 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 4.55 (s, D_2O exch., 1H, -OH), 7.00–7.96 (m, 9H, ArH and -N*H*-OH), 8.87 (s, D_2O exch., 1H, =NNH-), 10.24 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 404 [M^+ + 1] (16.9), 403 [M^+ + 1] (11.0), 402 [M^+] (48.8), 135 (100). Anal. calcd. for

$C_{17}H_{15}ClN_6O_2S$: C, 50.68; H, 3.75; N, 20.86; S, 7.96. Found: C, 50.47; H, 3.83; N, 20.72; S, 8.11.

N-Hydroxy-2-(2-(benzothiazol-2-oyl)hydrazono)-N′-(4-nitrophenyl)propanehydrazonamide ***6b***

Orange fibers, yield (56%); m.p.: >300°C; IR (KBr) ν_{max} cm^{-1}: 3395–3130 (3 NH + OH), 1682 (C=O), 1586 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 5.04 (s, D_2O exch., 1H, -OH), 6.97–8.08 (m, 9H, ArH and -N*H*-OH), 9.11 (s, D_2O exch., 1H, =NNH-), 10.65 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 413 [M^+] (26.8), 135 (100). Anal. calcd. for $C_{17}H_{15}N_7O_4S$: C, 49.39; H, 3.66; N, 23.72; S, 7.76. Found: C, 49.50; H, 3.59; N, 23.73; S, 7.64.

General procedure for synthesis of (1E,2Z,3E)-1-(piperidin-1-yl)-1-(arylhydrazono)-2-[(benzoyl/benzothiazol-2-oyl)hydrazono]-4-(aryl^1)but-3-enes ***7a–g***

To a solution of propanehydrazonoyl chloride **3a–e** (1 mmol) in ethanol (20 mL), piperidine (0.34 g, 4 mmol) and the appropriate aldehyde (1 mmol) were added. The reaction mixture was refluxed for 8 h. The precipitated product was filtered off. Recrystallization from EtOH/DMF afforded compounds **7a–g**, respectively.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(phenylhydrazono)-2-[(benzoyl)hydrazono]-4-(phenyl)but-3-ene ***7a***

Yellow crystals, yield (64%); m. p.: 240–242°C; IR (KBr) ν_{max} cm^{-1}: 3384, 3235 (2 NH), 1683 (C=O), 1601 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.51 (m, 6H, 3 CH_2 of piperidine), 3.45 (m, 4H, 2 CH_2 of piperidine), 6.69–7.98 (m, 17H, ArH and olefinic protones), 9.02 (s, D_2O exch., 1H, =NNH-), 10.81 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 452 [M^+ + 1] (2.9), 451 [M^+] (7.4), 84 (100). Anal. calcd. for $C_{28}H_{29}N_5O$: C, 74.47; H, 6.47; N, 15.51. Found: C, 74.33; H, 6.54; N, 15.60.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(phenylhydrazono)-2-[(benzoyl)hydrazono]-4-(4-methoxyphenyl) but-3-ene ***7b***

Yellow crystals, yield (66%); m. p.: 236–238°C; IR (KBr) ν_{max} cm^{-1}: 3393, 3219 (2 NH), 1682 (C=O), 1603 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.52 (m, 6H, 3 CH_2 of piperidine), 3.64 (m, 4H, 2 CH_2 of piperidine), 3.77 (s, 3H, OCH_3), 6.71–7.92 (m, 16H, ArH and olefinic protones), 9.52 (s, D_2O exch., 1H, =NNH-), 10.65 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 481 [M^+] (9.8), 77 (100). Anal. calcd. for $C_{29}H_{31}N_5O_2$: C, 72.33; H, 6.49; N, 14.54. Found: C, 72.50; H, 6.45; N, 14.67.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(4-tolylhydrazono)-2-[(benzoyl)hydrazono]-4-(phenyl)but-3-ene ***7c***

Yellow crystals, yield (68%); m. p.: 249–251°C; IR (KBr) ν_{max} cm^{-1}: 3266–3187 (2 NH), 1639 (C=O), 1571 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.61 (m, 6H, 3 CH_2 of piperidine), 2.34 (s, 3H, CH_3), 3.30 (m, 4H, 2 CH_2 of piperidine), 6.0–8.0 (m, 16H, ArH and olefinic protones), 9.43 (s, D_2O exch., 1H, =NNH-), 10.75 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 465 [M^+] (4.4), 84 (100). Anal. calcd. for $C_{29}H_{31}N_5O$: C, 74.81; H, 6.71; N, 15.04. Found: C, 74.57; H, 6.59; N, 14.86.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(4-tolylhydrazono)-2-[(benzoyl)hydrazono]-4-(thein-2-yl)but-3-ene ***7d***

Yellow crystals, yield (73%); m.p.: 218–220°C; IR (KBr) ν_{max} cm^{-1}: 3287–3170 (2NH), 1668 (C=O), 1585 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.60 (m, 6H, 3 CH_2 of piperidine), 2.33 (s, 3H, CH_3), 3.32 (m, 4H, 2 CH_2 of piperidine), 6.96–8.02 (m, 14H, ArH and olefinic protones), 8.89 (s, D_2O exch., 1H, =NNH-), 10.88 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 471 [M^+] (17.3), 84 (100). Anal. calcd. for $C_{27}H_{29}N_5OS$: C, 68.76; H, 6.20; N, 14.85; S, 6.80. Found: C, 68.58; H, 6.04; N, 14.94; S, 6.63.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(phenylhydrazono)-2-[(benzothiazol-2-oyl)hydrazono]-4-(phenyl) but-3-ene ***7e***

Yellow crystals, yield (75%); m. p.: 221–223°C; IR (KBr) ν_{max} cm^{-1}: 4012–3193 (2 NH), 1686 (C=O), 1598 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.63 (m, 6H, 3 CH_2 of piperidine), 3.30 (m, 4H, 2 CH_2 of piperidine), 6.92–7.99 (m, 14H, ArH and olefinic protones), 8.97 (s, D_2O exch., 1H, =NNH-), 10.86 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 509 [M^+ + 1] (4.2), 508 [M^+] (4.6), 84 (100). Anal. calcd. for $C_{29}H_{28}N_6OS$: C, 68.48; H, 5.55; N, 16.52; S, 6.30. Found: C, 68.25; H, 5.39; N, 16.70; S, 6.09.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(4-chlorophenyl-hydrazono)-2-[(benzothiazol-2-oyl)hydrazono]-4-(phenyl)but-3-ene ***7f***

Yellow crystals, yield (67%); m. p.: 239–241°C; IR (KBr) ν_{max} cm^{-1}: 3384, 3235 (2 NH), 1683 (C=O), 1601 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.52 (m, 6H, 3 CH_2 of piperidine), 3.29 (s, 3H, OCH_3), 3.30 (m, 4H, 2 CH_2 of piperidine), 6.87–8.08 (m, 15H, ArH and olefinic protones), 8.96 (s, D_2O exch., 1H, =NNH-), 10.47 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 545 [M^+ + 2] (5.8), 544 [M^+ + 1] (4.5), 543 [M^+] (13.9), 84 (100). Anal. calcd. for $C_{29}H_{27}ClN_6OS$: C, 64.14; H, 5.01; N, 15.47; S, 5.90. Found: C, 63.96; H, 4.94; N, 15.34; S, 5.97.

(1E,2Z,3E)-1-(Piperidin-1-yl)-1-(4-nitrophenyl-hydrazono)-2-[(benzothiazol-2-oyl)hydrazono]-4-(phenyl)but-3-ene ***7g***

Yellow crystals, yield (69%); m.p.: 272–274°C; IR (KBr) ν_{max} cm^{-1}: 3370–3118 (2 NH), 1680 (C=O), 1589 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.60 (m, 6H, 3 CH_2 of piperidine), 3.25 (m, 4H, 2 CH_2 of piperidine), 6.87–8.11 (m, 13H, ArH and olefinic protones), 9.14 (s, D_2O exch., 1H, =NNH-), 11.70 (s, D_2O exch., 1H, -CONH-); MS *m/z* (%): 555 [M^+ + 2] (1.5), 554 [M^+ + 1] (2.9), 553 [M^+] (6.0), 84 (100). Anal. calcd. for $C_{29}H_{27}N_7O_3S$: C, 62.91; H, 4.92; N, 17.71; S, 5.79. Found: C, 62.75; H, 5.04; N, 17.70; S, 5.84.

X-ray crystallography

A single crystal of compound **7g** was obtained by slow evaporation at room temperature, from a mixture of ethanol/DMF (v/v = 5:1). The crystal structure was solved and refined using maxus (nonius, Deflt and MacScience, Japan) [46] Mo-Ka radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system is shown in Fig. 1. Crystal data for compound **7g**: $C_{28}H_{27}N_7O_3S$, M_r, 553.645; system, monoclinic; Space group, $P2_1/c$; unit cell dimensions, *a* 12.8288 (4) Å, *b* 11.2318 (4) Å, *c* 20.4612 (9) Å, α 90.00°, β 92.4035 (12)°; *V*, 2945.7 (2) Å^3; *Z*, 4; D_x, 1.248 mg m^{-3}; θ range for data collection, 2.910–27.485°; μ(Mo-Ka), 0.15 mm^{-1}; T, 298 K; measured reflections, 11328; independent reflections, 8615;

observed reflections, 1609; R_{int}, 0.038; *R(all)*, 0.313; *wR(ref)*, 0.083; *wR(all)*, 0.187; *S(ref)*, 1.827; *S(all)*, 1.976; Δ/σ_{max}, 0.045; $\Delta\rho_{max}$, 0.89 eÅ^3, $\Delta\rho_{min}$, − 1.03 eÅ^3. Crystallographic data for the structures **7g** have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 742663. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

Antiviral and cytotoxicity assays

Sample preparation

Samples were prepared for assay by dissolving in 50 mL of DMSO and diluting aliquots into sterile culture medium at 0.4 mg/mL. These solutions were subdiluted to 0.02 mg/mL in sterile medium and the two solutions used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg/mL in triplicate in the wells of microtiter plates.

Virus used in assay

The tested compounds were tested for antiviral activity against herpes simplex type 1 (HSV-1) grown on *Vero* African green monkey kidney cells.

Culture

Herpes simplex type 1 (HSV-1) was the gift of Dr. R. G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. USA. Virus stocks were prepared as aliquots of culture medium from *Vero* cells infected at a multiplicity of 1 virion per 10 cells and cultured for three days. They were stored at −80°C. Working stocks were prepared by titering virus by serial dilution in culture medium and assayed in triplicate on Vero monolayers in the wells of microtiter trays. Virus suspensions that gave about 30 plaques per well were stored at 4°C until used. *Vero* African green monkey kidney cells were purchased from Viromed Laboratories, Minnetonka, MN, USA, and grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum (HyClone Laboratories, Ogden, UT, USA), 60 mg/mL Penicillin G and 100 mg/mL streptomycin sulfate maintained at 37°C in a humidified atmosphere containing about 15% (v/v) CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. *Vero* stocks were maintained at 34°C in culture flasks filled with medium supplemented with 1% (v/v) calf serum. Subcultures for virus titration or antiviral screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton Dickinson Labware, Lincolin Park, NJ, USA) by suspending *Vero* cells in medium following trypsin-EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10% calf serum to 2 6 104 cells per 200 mL culture, aliquoting into each well of a tray and culturing until confluent.

Procedure

Microtiter trays with confluent monolayer cultures of *Vero* cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile compounds in triplicate in 100 μL medium followed by titered virus in 100 μL medium containing 10% (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were not treated with compounds or virus. The trays were cultured for 66 h. The trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined visually. Antiviral activity is identified as confluent, relatively unaltered monolayers of stained *Vero* cells treated with HSV-1. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HSV-1.

The authors have declared no conflict of interest.

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