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ORIGINAL PAPER

Methyl-2-arylidene hydrazinecarbodithioates: synthesis and biological activity

[a]Manojkumar Mahapatra, [b]Umasankar Kulandaivelu, [c]Philipp Saiko, [c]Geraldine Graser, [c]Thomas Szekeres, [d]Graciela Andrei, [d]Robert Snoeck, [d]Jan Balzarini, [a,e]Venkatesan Jayaprakash*

[a] *Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835 215, India*

[b] *Medicinal Chemistry Research Division, Vaagdevi College of Pharmacy, Hanamkonda, Warangal, Andhra Pradesh 506 001, India*

[c] *Department of Medical and Chemical Laboratory Diagnostics, General Hospital of Vienna – Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria*

[d] *Rega Institute for Medical Research, KU Leuven, B-3000, Leuven, Belgium*

[e] *Valens Pharma Services, Regus Citi Centre, Level 6, Chennai Citi Centre, 10/11, Dr.Radhakrishnan Salai, Chennai, Tamil Nadu 600 004, India*

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Methyl-2-arylidene hydrazine-carbodithioate has not been of particular interest to researchers even though its metal complexes are extensively reported on due to their biological activity. This study examined the cytostatic and antiviral activity of twelve methyl-2-arylidene hydrazinecarbodithioates reported by many researchers as intermediates for the synthesis of thiosemicarbazides and the preparation of their metal complexes. Compounds *IIc*, *IIi*, and *III* with tridentate ligand features were found to have the lowest IC_{50} value (6.5 μM, ≈ 1 μM, and 0.8 μM, respectively) against HL60 human promyelocytic leukemia cells. They were also most inhibitory to human embryonic lung (HEL) fibroblast proliferation (5.3 μM, 17 μM, and 2.6 μM). Compound *IIc* and *III* show antiviral activity against wild-type herpes simplex virus (HSV), varicella zoster virus (VZV), and acyclovir-resistant HSV; however, these activities were observed at concentrations at which the compounds also markedly inhibit HL60 and HEL cell proliferation.

Keywords: Schiff's base, methyl hydrazinecarbodithioate, HL60 cell line, anticancer, antiviral, cytotoxicity

Introduction

Many N-substituted thiosemicarbazones were prepared by the condensation of Schiff's bases of methyl-2-arylidene hydrazinecarbodithioate (MAHCD) with amines (Casero et al., 1980; Collins et al., 1982; Klayman et al., 1979, 1991; Scovill et al., 1982; Shipman et al., 1981). While thiosemicarbazones and their metal complexes were studied for their biological activity (Beraldo & Gambinob, 2004; Ettari et al., 2010; Jiang et al., 2006; Katz, 1987; Liberta & West, 1992; Matesanz & Souza, 2009; Pandeya & Dimmock, 1993; Wnuk & Robins, 2006; Yu et al., 2009), the intermediate MAHCD was rarely studied for its metal complexation behaviour and the complexes for their biological activity (Ali et al., 2002; Kanwar et al.,

*Corresponding author, e-mail: venkatesanj@bitmesra.ac.in

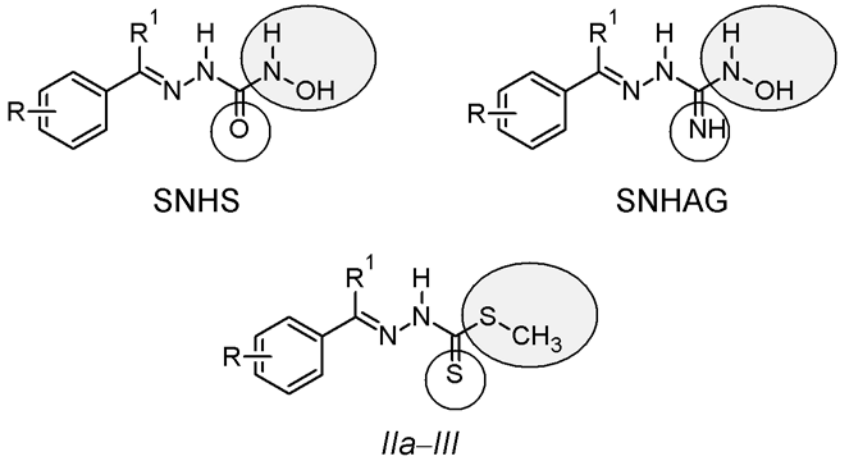


Fig. 1. Similarity of title compounds with Schiff's base of *N*-hydroxy semicarbazides (SNHS) and *N*-hydroxy aminoguanidines (SNHAG).

2008; Neelam et al., 2000; Saxena & Tandon, 1983; Singh et al., 1997). Very few investigators have reported biological activity of compounds with a dithiocarbamate side chain (Cao et al., 2005; Huang et al., 2009; Kumar et al., 2010). It is interesting to note that MAHCD shares structural similarity with Schiff's bases of *N*-hydroxy semicarbazides (SNHS) and *N*-hydroxy aminoguanidines (SNHAG) (Fig. 1). Both SNHS and SNHAG were rigorously documented for their anticancer activity and antiviral properties. It was proposed that these compounds act by inhibiting ribonucleotide reductase (RR) through metal chelation (Das et al., 1999; Ren et al., 2002; T'ang et al., 1985). With this background, twelve MAHCD derivatives were synthesised and evaluated for anticancer activity against HL60 human promyelocytic leukemia cells and antiviral activity against a panel of viral cell lines.

Experimental

Synthesis of methyl hydrazinecarbodithioate (I) and substituted benzaldehyde hydrazones of methyl hydrazine carbodithioate (II)

To an ice cooled solution (< 10 °C) of 19.8 g (0.300 mol) of potassium hydroxide in 24 mL of water and 20 mL of propan-2-ol, 17.1 mL of 80 % pure hydrazine hydrate were added and constantly stirred. The amount of 18.2 mL (0.300 mol) of ice cooled carbon disulfide was added drop wise to the stirred solution, maintained at < 10 °C for over about 1–1.5 h. The bright yellow mixture formed was stirred for an additional 1 h after which ice cooled iodomethane (18.7 mL, 0.300 mol) was added drop wise over a 2 h period. Stirring was continued for an additional 1 h, and the white precipitate obtained was filtered and washed with ice-cold water. The crude product was recrystallised from dichloromethane: yield of 43 %; m.p. = 82 °C. (82 °C, Audrieth et al. (1954); 81–83 °C, Klayman et al. (1979)).

Methyl hydrazinecarbodithioate (20.0 mmol) was dissolved in 20 mL of methanol and then, equimolar amount of aromatic/heteroaromatic aldehyde was added. The mixture was refluxed for 6–12 h and the reaction was monitored by TLC. The hot solution was poured onto crushed ice and the precipitate obtained was filtered (Klayman et al., 1979; Scovill et al., 1982): yield of 60–80 %.

Anticancer activity assay procedure

The HL-60 human promyelocytic leukemia cell line was purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were grown in a RPMI 1640 medium supplemented with a 10 % heat inactivated fetal calf serum (FCS), 1 % L-glutamine and 1 % penicillin–streptomycin in a humidified atmosphere containing 5 % of CO_2. All media and supplements were obtained from Life Technologies (Paisley, Scotland, UK). Cell counts were determined using a micro cell counter CC-108 (SYSMEX, Kobe, Japan).

Cells growing in the logarithmic phase of growth were used in all experiments described below. HL-60 cells (10^5 per mL) were seeded in 25 cm^2 Nunc tissue culture flasks and incubated with increasing concentrations of drugs (*IIa–IIl*) at 37 °C under cell culture conditions. Cell counts and IC_{50} values were determined after 24 h, 48 h, and 72 h using the micro cell counter CC-108. Viability of the cells was determined by trypan blue exclusion. Results were calculated as the number of viable cells.

Antiviral activity assay procedure

Antiviral assays (except anti-human immunodeficiency virus (HIV) assays) were based on the inhibition of virus-induced cytopathic effect in HEL

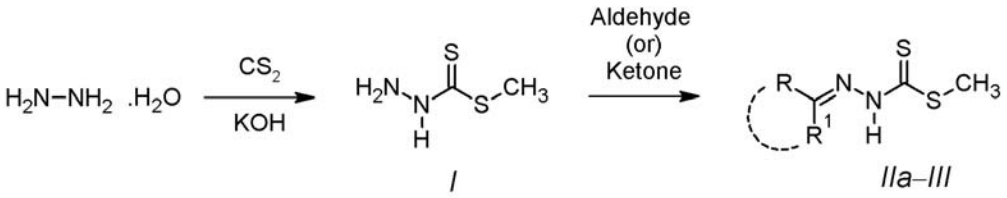


Fig. 2. Reaction sequence of the *IIa–Ill* synthesis.

(herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus), Vero (parainfluenza-3, reovirus-1, Coxsackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus), Madin-Darby canine kidney (MDCK) (influenza A (H1N1; H3N2) and B virus), and CrFK (feline corona virus (FIPV) and feline herpes virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100-cell culture inhibitory dose-50 ($CCID_{50}$) of the virus (1 $CCID_{50}$ being the virus dose to infect 50 % of the cells) in the presence of varying concentrations (100 mM, 20 mM, 5 mM, 1 mM, 200 nM) of the test compounds. Viral cytopathic effect was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

For the HCMV assays, confluent HEL fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus strains Davis and AD-169 at 100 PFU per well. After a 2 h incubation period, the residual virus was removed and the infected cells were further incubated with a medium containing different concentrations of the test compounds (in duplicate). After incubation for 7-days at 37 °C, the virus-induced cytopathic effect was monitored microscopically after ethanol fixation and staining with Giemsa dye. Antiviral activity was expressed as the EC_{50} or compound concentration required for the reduction of virus-induced cytopathogenicity by 50 %.

The varicella zoster virus (VZV) drug susceptibility tests were performed on confluent HEL cells in 96-well microtiter plates by the plaque reduction assay. Monolayers were infected with 20 plaque forming units (PFU) of the cell-associated virus per well. For each assay, virus controls (infected-untreated cells) were included. After a 2 h incubation period, the virus inoculum was removed and the media were replaced by different dilutions (in duplicate) of the tested molecules. Serial dilutions of test compounds were incubated with the infected monolayers for five days. After a five day incubation period, the cells were fixed and stained with Giemsa, and the level of the virus-induced cytopathic effect was determined by counting the number of plaques for each dilution. Activity was expressed as EC_{50} (effective compound concentration required to reduce virus plaque formation by 50 %) compared to the untreated control.

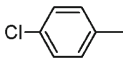
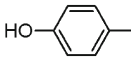
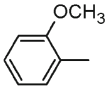
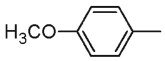
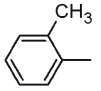
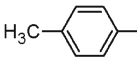
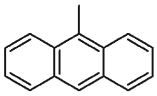
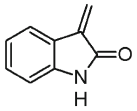
The anti-HIV activity of the compounds was evaluated against the wild type HIV-1 strain IIIB in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50 % cell culture infective dose (CCID50). MT-4 cells were suspended in culture medium at 10^5 cells per mL and infected with HIV at the multiplicity of infection of 0.02. Immediately after viral infection, 100 μL of the cell suspension were placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in DMSO at 50 mM or more as stock solutions. Then, serial dilutions of the test compounds were made. The highest concentration tested was 100 μM of the test compound containing 0.2 % of DMSO, which is by itself not toxic to cell proliferation. After four days of incubation at 37 °C, the number of viable cells was determined using the MTT method.

Results and discussion

Compounds *IIa–Ill* were synthesised following the reaction sequence outlined in Fig. 2. Methyl hydrazinecarbodithioate (*I*) was prepared by the reaction of hydrazine hydrate (85 mass %) with carbon disulfide in the presence of potassium hydroxide at a temperature below 10 °C; followed by the addition of methyl iodide (Audrieth et al., 1954). Condensation of *I* with aromatic aldehydes/ketones in methanol provided compounds *IIa–Ill* (Klayman et al., 1991; Scovill etal., 1982). All compounds were characterised by their ^{1}H-NMR and FAB-MS data. Physicochemical characterisation data are presented Table 1 and spectral data of compounds *IIa–Ill* are presented in Table 2.

All compounds were tested for their anticancer activity against the HL-60 human promyelocytic leukemia cell line and the results are presented in Table 3. The compounds were also tested for their antiviral activity against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 TK^- (KOS ACV^r), varicella-zoster virus (OKA and 07/1), cytomegalovirus (Davis and AD169), vaccinia virus, vesicular stomatitis virus, feline corona virus, feline herpes virus, human immunodeficiency virus (HIV) type 1 (III_B) and type 2 (ROD), coxsackie virus B4, respiratory syncytial virus, parainfluenza 3 virus, re-

Table 1. Characterisation data of newly prepared compounds *IIa–IIl*

Compound	R	R^1	Formula	M_r	Yield / %	M.p. / °C
IIa		H	$C_9H_9ClN_2S_2$	244	67	187
IIb		H	$C_9H_9ClN_2S_2$	244	72	165
IIc		H	$C_9H_{10}N_2OS_2$	226	61	178
IId		H	$C_9H_{10}N_2OS_2$	226	60	139
IIe		H	$C_{10}H_{12}N_2OS_2$	240	62	143
IIf		H	$C_{10}H_{12}N_2OS_2$	240	75	141
IIg		H	$C_{10}H_{12}N_2S_2$	224	80	118
IIh		H	$C_{10}H_{12}N_2S_2$	224	69	153
IIi		CH_3	$C_{10}H_{12}N_2OS_2$	240	63	162
IIj		H	$C_{17}H_{16}N_2S_2$	312	61	189
IIk		–	$C_{10}H_9N_3OS_2$	251	69	181
IIl		H	$C_8H_9N_3S_2$	211	63	143

ovirus, sindbis virus and punta tora virus. Only results for compounds showing activity against any of the viruses are presented in Table 4.

Compound *IIl*, *IIi*, and *IIc* showed IC_{50} values against HL60 cells at the concentration of 0.8 μM, ≈ 1 μM, and 6.5 μM, respectively, after 72 h. It is interesting to note that preferably compounds with tridentate ligand characteristics (*IIl*, *IIi*, and *IIc*) showed potent anti-proliferative activity against the HL60 cells (Table 3). The activity of compound *IIi* (2′-hydroxy acetophenone derivative) was higher than that of *IIc* (2-hydroxy benzaldehyde derivative) and almost equally potent to *IIl* (pyridine 2-carboxaldehyde derivative). All the other compounds (*IIa*, *IIb*, *IId–IIh*, and *IIk*) were inhibitory at concentrations between 55 μM and 63 μM after 72 h, except for *IIj*, irrespective of the nature and position of the substitution. Compound *IIj* did not exert any inhibitory activity on the HL60 cells at the maximum concentration studied (100 μM, after 72 h). All the compounds exhibited a cytotoxic concentration (CC_{50}) of ≥ 100 μM in the HeLa cell cultures except for *IIl* (36 μM), (Table 3). Compounds *IIi* and *IIl* showed the best selectivity towards the HL60 cells and therefore, further investigations are required.

Compounds *IIc* and *IIi* resemble the iron chelator 311 (*N*′-[(2-hydroxynaphthalen-1-yl)-methylidene]-pyridine-4-carbohydrazide) and *IIl* resembles triapine (3-aminopyridine-2-carbaldehyde thiosemicarbazone). Compound *IIi* having a ketonic methyl group showed activity comparable to that of iron chelator 311 at 48 h. Compound *IIl* also exhibited an anti-proliferative activity comparable to that of Triapine. The analogue of *IIl* with a ketonic methyl group has to be considered for further improvement of its activity.

It is interesting to observe that only compounds with tridentate ligand characteristics (*IIc*, *IIi*, and *IIl*) have shown antiviral activity against a few viruses and

Table 2. Spectral data of newly prepared compounds *IIa–Ill*

Compound	Spectral data
IIa	^{1}H NMR (DMSO-d_6), δ: 2.68 (s, 3H, —SCH_3); 7.41–7.87 (m, 4H, ArH); 8.41 (s, 1H, —N═CH—); 12.63 (s, 1H, NH) MS, m/z (I_r/%): 244 (21.60) $(M)^+$, 245 (63.42) $(M + 1)^+$, 246 (16.98) $(M + 2)^+$
IIb	^{1}H NMR (DMSO-d_6), δ: 2.75 (s, 3H, —SCH_3); 7.26–7.68 (m, 4H, ArH); 7.83 (s, 1H, —N═CH—); 10.3 (s, 1H, NH) MS, m/z (I_r/%): 244 (22.04) $(M)^+$, 245 (61.04) $(M + 1)^+$, 246 (16.92) $(M + 2)^+$
IIc	^{1}H NMR (DMSO-d_6), δ: 2.66 (s, 3H, —SCH_3); 6.92–7.42 (m, 4H, ArH); 8.33 (s, 1H, —N═CH—); 10.44 (s, 1H, NH); 13.04 (s, 1H, ArOH) MS, m/z (I_r/%): 226 (20.30) $(M)^+$, 227 (68.61) $(M + 1)^+$, 228 (11.09) $(M + 2)^+$
IId	^{1}H NMR (DMSO-d_6), δ: 2.73 (s, 3H, —SCH_3); 6.82–7.95 (m, 4H, ArH); 8.14 (s, 1H, —N═CH—); 10.06 (s, 1H, NH); 13.13 (s, 1H, ArOH) MS, m/z (I_r/%): 226 (44.77) $(M)^+$, 227 (42.71) $(M + 1)^+$, 228 (12.52) $(M + 2)^+$
IIe	^{1}H NMR (DMSO-d_6), δ: 2.67 (s, 3H, —SCH_3); 3.88 (s, 3H, —OCH_3); 6.90–7.99 (m, 4H, ArH); 8.29 (s, 1H, —N═CH—); 10.17 (s, 1H, NH) MS, m/z (I_r/%): 240 (24.83) $(M)^+$, 241 (58.85) $(M + 1)^+$, 242 (16.32) $(M + 2)^+$
IIf	^{1}H NMR (DMSO-d_6), δ: 2.67 (s, 3H, —SCH_3); 3.86 (s, 3H, —OCH_3); 6.72–7.69 (m, 4H, ArH); 7.79 (s, 1H, —N═CH—); 10.06 (s, 1H, NH) MS, m/z (I_r/%): 240 (37.65) $(M)^+$, 241 (43.62) $(M + 1)^+$, 242 (18.73) $(M + 2)^+$
IIg	^{1}H NMR (DMSO-d_6), δ: 2.53 (s, 3H, CH_3); 2.67 (s, 3H, —SCH_3); 7.26–7.80 (m, 4H, ArH); 8.12 (s, 1H, —N═CH—); 10.36 (s, 1H, NH) MS, m/z (I_r/%): 224 (23.63) $(M)^+$, 225 (57.06) $(M + 1)^+$, 226 (19.30) $(M + 2)^+$
IIh	^{1}H NMR (DMSO-d_6), δ: 2.39 (s, 3H, CH_3); 2.67 (s, 3H, —SCH_3); 7.21–7.63 (m, 4H, ArH); 7.84 (s, 1H, —N═CH—); 10.27 (s, 1H, NH) MS, m/z (I_r/%): 224 (25.05) $(M)^+$, 225 (60.48) $(M + 1)^+$, 226 (14.47) $(M + 2)^+$
IIi	^{1}H NMR (DMSO-d_6), δ: 2.42 (s, 3H, CH_3); 2.73 (s, 3H, —SCH_3); 6.94–7.53 (m, 4H, ArH); 9.93 (NH); 11.37 (s, 1H, ArOH) MS, m/z (I_r/%): 240 (22.82) $(M)^+$, 241 (54.35) $(M + 1)^+$, 242 (22.82) $(M + 2)^+$
IIj	^{1}H NMR (DMSO-d_6), δ: 2.50 (s, 3H, —SCH_3); 7.56–8.38 (m, 9H, ArH); 8.66 (s, 1H, —N═CH—); 11.32 (NH) MS, m/z (I_r/%): 95 (26.20), 107 (27.99), 204 (19.91), 221 (25.86)
IIk	^{1}H NMR (DMSO-d_6), δ: 2.67 (s, 3H, —SCH_3); 7.2–7.9 (m, 4H, ArH); 8.2 (s, 1H, NH); 8.9 (s, 1H, —CONH—) MS, m/z (I_r/%): 251 (23.29) $(M)^+$, 252 (44.98) $(M + 1)^+$, 253 (31.73) $(M + 2)^+$
Ill	^{1}H NMR (DMSO-d_6), δ: 2.63 (s, 3H, —SCH_3); 6.5–7.4 (m, 4H, ArH); 8.3 (s, 1H, NH) MS, m/z (I_r/%): 211 (20.80) $(M)^+$, 212 (48.32) $(M + 1)^+$, 213 (30.87) $(M + 2)^+$

Table 3. Cytotoxic/cytostatic activity of compounds *IIa–Ill* against HeLa and HL60 cells

Code	HeLa cells	HL60 cells			
	CC_{50}^a	IC_{50} (24 h)	IC_{50} (48 h) μM	IC_{50} (72 h)	IC_{50} (96 h)
IIa	100	≈ 90	≈ 60	≈ 60	–
IIb	> 100	> 100	70	62.5	–
IIc	> 100	> 100	6.5	6.5	–
IId	> 100	97	65	63	–
IIe	100	> 100	59	55	–
IIf	>100	≈ 85	≈ 60	≈ 60	–
IIg	> 100	96	59	59	–
IIh	100	96	69	57	–
IIi	100	≈ 85	≈ 1	≈ 1	–
IIj	100	> 100	> 100	> 100	–
IIk	> 100	93	64	57	–
Ill	35.8	1	0.8	0.8	–
Iron chelator 311[b]	–	0.6	0.4	–	–
TRP[c]	–	–	–	–	0.27

a) 50 % cytotoxic concentration as determined by measuring the cell viability with the colorimetric formazan-based MTS assay; *b*) values are taken from Richardson and Milnes (1997), iron chelator 311 = (*N′*-[(2-hydroxynaphthalen-1-yl)-methylidene]-pyridine-4-carbohydrazide); *c*) values are taken from Kowol et al. (2009); TRP = triapine (triapine = 3-aminopyridine-2-carbaldehyde thiosemicarbazone).

are presented in Table 4. Compound *Ill* displayed pronounced antiviral activity against HSV-1 (8–42 μM), HSV-2 (9–11 μM), VZV (6.5–42 μM), and vaccinia virus (15–45 μM). It should be noted that *Ill* inhibits

Table 4. Antiviral activity of compounds *IIc*, *IIi*, and *IIl* in HEL cell cultures

Code	EC_{50}^{a}									MCC[b]	CC_{50}^{c}
	HSV-1 (KOS)	HSV-2 (G)	Vaccinia virus	Vesicular stomatitis virus	HSV-1 TK$^-$ (KOS ACV^r)	VZV		CMV			
						OKA	07-1	Davis	AD169		
	μM										
IIc	10 ± 2	11 ± 1	12 ± 0	> 100	≥ 20	24	42	20	> 4	> 100	5.3
IIi	45 ± 0	≥ 100	45 ± 0	> 100	≥ 100	42	42	> 20	> 20	100	17
IIl	8 ± 1	9 ± 0	15 ± 6	> 20	≥ 20	6.5	11	> 4	> 4	≥ 20	2.6
Brivudin	0.1	183	29	> 250	250	0.01	28	–	–	> 200	> 200
Acyclovir	1	0.4	250	> 250	146	0.8	89	–	–	> 200	> 200
Ganciclovir	0.03	0.03	100	> 100	20	–	–	6.3	3.1	> 200	> 200

a) Compound concentration required to reduce virus-induced cytopathogenicity by 50 %; *b*) compound concentration required to cause a microscopically detectable alteration of normal cell morphology; *c*) compound concentration required to inhibit HEL cell proliferation by 50 %.

the wild-type as well as the acyclovir-resistant HSV at comparable compound concentrations (8–20 μM, Table 4). Compound *IIc* with a 2-hydroxy substitution and compound *IIi*, an acetophenone analogue of *IIc*, show antiviral activity at somewhat higher concentrations than *IIl*. Compound *IId* that differs from *IIc* by a hydroxy functional group at the *para* position did not show any appreciable antiviral activity. Additional analogues of *IIc* and *IIl* are currently under further investigation. It should, however, be noted that *IIc*, *IIi*, and *IIl* are poorly cytotoxic but they show pronounced cytostatic activity in proliferating HEL (and HL60) cell cultures. Therefore, the observed antiviral activity might not be due to direct antiviral activity but rather due to indirect inhibitory action caused by the underlying cytostatic potential of these compounds.

Conclusions

The present investigation revealed that: *i*) compounds with a metal chelating functional group (*IIc*, *IIi*, and *IIl*) at their aryl end were found to be active and *ii*) the methyl dithioate (—$CSSCH_3$) group is not an bioisosteric equivalent of hydroxamate (—CONHOH) or amidoxime (—CNHNHOH) (Fig 1.). The compounds investigated are known as intermediates in the synthesis of many thiosemicarbazones but their usefulness as medicinally active agents has not yet been studied. The study suggests a possibility of intermediates with specific pharmacophoric features for the intended activities that can provide a new scaffold for these activities.

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