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Synthesis and studies of novel homoveratryl based thiohydantoins as antibacterial as well as anti-HIV agents

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N,N′-bis(3,4-dimethoxyphenylethyl)-5-(arylidene)-2-thiohydantoins **3a-j** have been prepared by condensation of 1,3-diaryl-2-thiohydantoin 2 with various aromatic aldehydes. The compound **2** has been prepared by the reaction of N,N′-bis(3,4-dimethoxyphenylethyl)thiourea **1** with chloroaceticacid. The compounds have been tested for their antibacterial and anti-HIV activities against different microorganisms. The structures of novel synthesized compounds have been established on the basis of elemental analyses, ^{1}H NMR, IR and mass spectral data.

Keywords: Homoveratrylamine, thiohydantoin, styrylation, antibacterial activity, anti-HIV activity

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The thiohydantoin nucleus is a 5-membered ring system containing a reactive cyclic thiourea core. This heterocycle is present in a wide range of biologically active compounds including antiarrhythmic[1], anti-convulsant[2] and antitumor[3] agents. 2-Thiohydantoin[4,5] moiety possesses antiviral, antibacterial, antifungal, herbicidal, anticonvulsant, antidiabetic, anti-inflammatory, antiulcer and antiarrhythmic properties. A series of 2-thiohydantoins carrying various heterocyclic substituents such as 5-bromothienylidene, 5-(2-carboxyphenylthio)-2-thienylidene and 4*H*-thieno-[2,3-*b*][1] benzothiopyran-4-one have been synthesized and tested for their activity against HIV-1 virus and as antitumor agents[6].

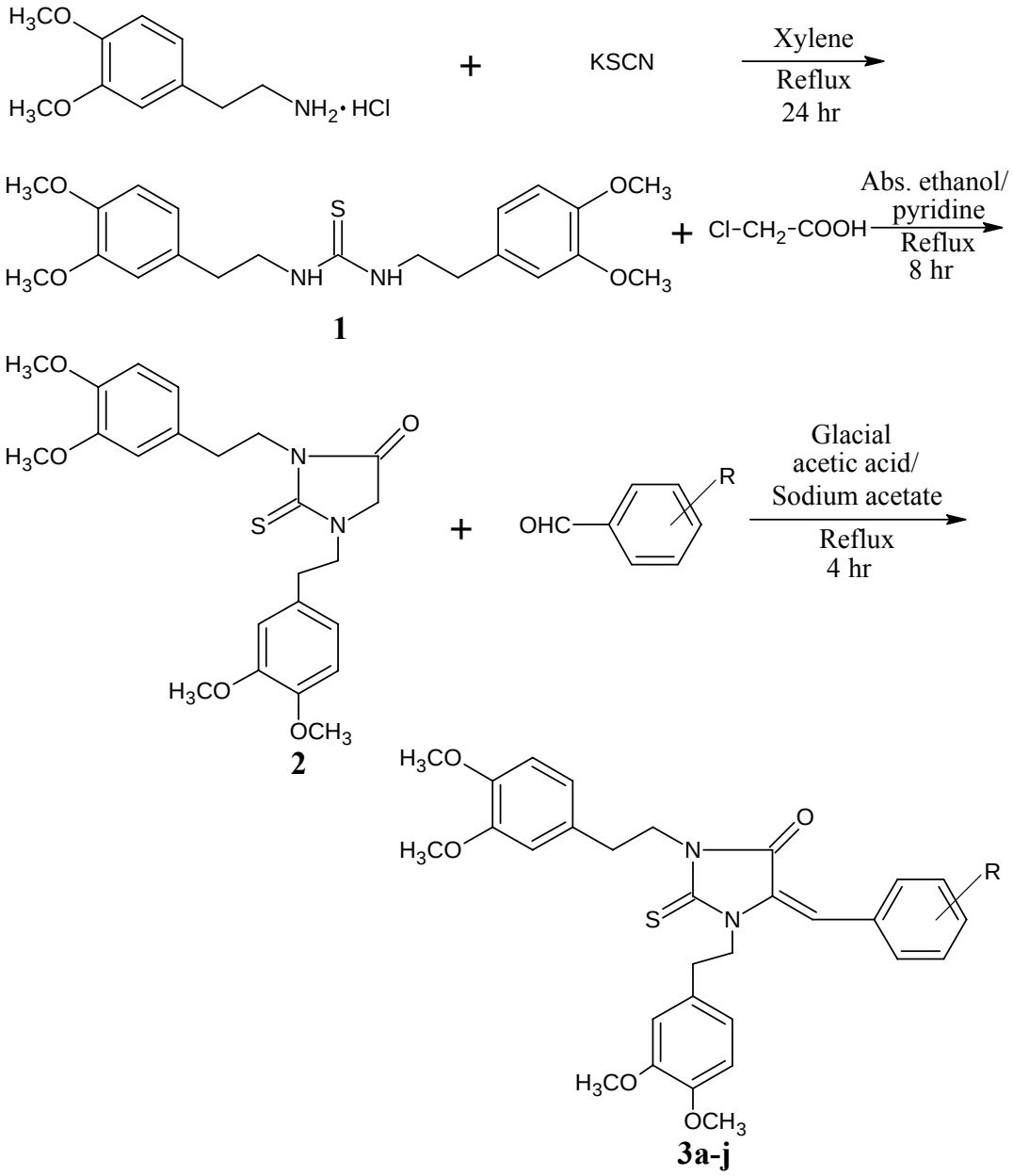
Thiourea derivatives of phenylethyl amine are used in the synthesis of anti-HIV[7] compounds, pig kidney aldose reductase[8], and antischizophrenics[9]. Moreover, the chloroacetyl derivatives possess local anaesthetic[10], herbicidal[11], antibiotic[12], anticancer, anti-HIV[13], spasmolytic and antiepileptic[14], 5-HT_{1A} antagonist[15], antitumour[16], antibacterial[17] and antifungal[18] properties.

Based on the above observations, it was decided to synthesize novel thiohydantoin derivatives having ethyl linked 3,4-dimethoxyphenylethyl thiourea derivatives with styryl bridge. Thus, novel N,N′-bis(3,4-dimethoxyphenylethyl)-5-(arylidene)-2-thiohydantoins (**3a-j**, **Scheme I**, **Table I**) were prepared by reacting N,N′-bis(3,4-dimethoxyphenylethyl)thiourea **1** and chloroacetic acid followed by condensation with different aromatic aldehydes[19]. Compound **1** was prepared by refluxing 3,4-dimethoxyphenylethyl hydrochloride and KSCN in xylene[20].

Experimental Section

Melting points were determined on an electro thermal apparatus (capillary method) and are uncorrected. IR spectra were recorded on a BOMMEN FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer using TMS as internal standard (chemical shift in δ ppm). Elemental analyses were done on Haraeus Rapid Analyser.

N,N′-Bis(3,4-dimethoxyphenylethyl)thiourea 1 (Ref. 20). To a stirred solution of 3,4-dimethoxy-phenylethyl amine hydrochloride (0.05 mole, 10.88 g) in xylene (100 mL) was added KSCN (0.025 mole, 2.4 g). The mixture was maintained at reflux for 24 h at which point TLC analysis (1:1 EtOAc/hexane) indicated the complete consumption of the starting material. The mixture was cooled to RT, diluted with H_2O (100 mL) and extracted with EtOAc (2×100 mL), collecting the emulsion along with the organic phase. The interfacial solid material and the EtOAc



Scheme I

Table I – Characterization data of the compounds **3a-j**

Compd	R	Mol. formula	m.p. °C	Yield %	Elemental analyses Found % (Calcd) C	H	N
3a	H	$C_{30}H_{32}N_2O_5S$	Limpid	50	67.29 (67.67	5.85 6.01	5.15 5.26)
3b	3,4,5-$(OCH_3)_3$	$C_{33}H_{38}N_2O_8S$	120-22	57	63.33 (63.67	5.95 6.11	4.40 4.50)
3c	4-$N(CH_3)_2$	$C_{32}H_{37}N_3O_5S$	110-12	72	66.40 (66.78	6.35 6.43	7.21 7.30)

—Contd

Table I – Characterization data of the compounds **3a-j**—*Contd*

Compd	R	Mol. formula	m.p. °C	Yield %	Elemental analyses Found % (Calcd) C	H	N
3d	4-F	$C_{30}H_{31}N_2O_5SF$	75-80	66	65.22 (65.45	5.55 5.63	4.95 5.09)
3e	2-C_4H_3O	$C_{28}H_{30}N_2O_6S$	90-7	54	64.10 (64.37	5.50 5.74	5.26 5.36)
3f	4-OCH_3	$C_{31}H_{34}N_2O_6S$	140-42	65	66.00 (66.19	5.95 6.05	4.88 4.98)
3g	2-Cl	$C_{30}H_{31}N_2O_5SCl$	84-6	69	63.41 (63.54	5.38 5.48	4.84 4.94)
3h	3-Cl	$C_{30}H_{31}N_2O_5SCl$	100-02	60	63.44 (63.54	5.37 5.48	4.85 4.94)
3i	3-Br	$C_{30}H_{31}N_2O_5SBr$	107-09	70	58.80 (58.91	5.00 5.07	4.47 4.58)
3j	3-OC_6H_5	$C_{36}H_{36}N_2O_6S$	Limpid	63	69.10 (69.23	5.60 5.77	4.40 4.49)

extract were combined, washed with 1*N* HCl (100 mL) and brine (50 mL). The organic phase was concentrated to afford yellow leaflets, which was purified by recrystallization from ethanol to afford yellow crystals of the title product 1. m.p. Yield 70%; m.p. 40°C. Found: C, 62.28; H, 6.89; N, 6.66; S, 7.85. $C_{21}H_{28}N_2O_4S$ requires C, 62.37; H, 6.93; N, 6.93; S, 7.92%.

N,N′-Bis(3,4-dimethoxyphenylethyl)-2-thiohydantoin 2 (Ref. 19). A mixture of N,N′-bis(3,4-dimethoxyphenylethyl)thiourea (0.01 mole), chloroacetic acid (0.015 mole) and pyridine (0.01 mole) in ethanol (7.5 mL) were heated on a water bath for 8 h. The mixture was cooled to RT and poured into ice water containing dilute HCl with constant stirring. The crude product was extracted with CH_2Cl_2 (2×50 mL). The extract was washed with 10% $NaHCO_3$ solution (25 mL) and dried over anhydrous Na_2SO_4. After evaporation of CH_2Cl_2, yellow limpid was obtained. Yield 65%. Found: C, 62.08; H, 6.24; N, 6.00; S, 7.11. $C_{23}H_{28}N_2O_5S$ requires C, 62.16; H, 6.30; N, 6.29; S, 7.21%.

N,N′-Bis(3,4-dimethoxyphenylethyl)-5-(arylidene)-2-thiohydantoins 3a-j (Ref. 19). A mixture of N,N′-bis(3,4-dimethoxyphenylethyl)-2-thiohydantoin (0.0025 mole), aryl aldehydes (0.0025 mole) and fused sodium acetate (0.00375 mole, 0.31 g) in glacial acetic acid (30 mL) was refluxed for 4 h. The resulting mixture was cooled, excess of acetic acid removed by distillation, and the residue poured into crushed ice and neutralized by adding 10% $NaHCO_3$ solution. The solid thus obtained was filtered, washed and purified by recrystallization from ethanol to give the desired product. The characterization data of the novel compounds **3a-j** are given in **Table I**.

The spectral data of the novel synthesized compounds 3a-o are given below.

3a: MS: m/z 532 (M^+); IR (KBr): 3092 (=C-H str.), 1518 (C=C str.), 754 (C-H def.), 1740 (C=O str.), 1550 (C=S str.), 2890 (=C-H str.), 1237 (C-O-C str. (asym.), 1029 (C-O-C str. (sym.), 2936 (C-H str.), 1458 cm^{-1} (C-H def.); ^{1}H NMR (DMSO-d_6): δ 2.74-3.24 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.71 (s, 6H, 2×-OCH_3), 7.00-8.2 (m, 11H, Ar-H).

3b: MS: m/z 622(M^+); IR (KBr): 3092 (=C-H str.), 1518 (C=C str.), 760 (C-H def.), 1740 (C=O str.), 1545 (C=S str.), 2895 (=C-H str.), 1237 (C-O-C str. (asym.), 1029 (C-O-C str. (sym.), 2936 (C-H str.), 1458 cm^{-1} (C-H def.); ^{1}H NMR (DMSO-d_6): δ 2.72-3.21 (m, 8H, 4×-CH_2), 4.52 (s, 1H, C=CH), 3.60 (s, 6H, 2×-OCH_3), 3.72 (s, 6H, 2×-OCH_3), 3.78 (s, 6H, 2×-OCH_3), 3.90 (s, 3H, -OCH_3), 7.10-8.0 (m, 8H, Ar-H).

3c: MS: m/z 575(M^+); ^{1}H NMR (DMSO-d_6): δ 2.70-3.20 (m, 8H, 4×-CH_2), 4.51 (s, 1H, C=CH), 3.60 (s, 6H, 2×-OCH_3), 3.72 (s, 6H, 2×-OCH_3), 2.84 (s, 6H, -$N(CH_3)_2$), 7.05-8.15 (m, 10H, Ar-H).

3d: MS: m/z 550(M^+); ^{1}H NMR (DMSO-d_6): δ 2.71-3.20 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.73 (s, 6H, 2×-OCH_3), 6.80-8.00 (m, 10H, Ar-H).

3e: MS: m/z 522 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.74-3.24 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62

(s, 6H, 2×-OCH_3), 3.71 (s, 6H, 2×-OCH_3), 6.67 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.90-8.2 (m, 6H, Ar-H).

3f: MS: m/z 562 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.73-3.23 (m, 8H, 4×-CH_2), 4.53 (s, 1H, C=CH), 3.65 (s, 9H, 3×-OCH_3), 3.72 (s, 6H, 2×-OCH_3), 6.90-7.90 (m, 10H, Ar-H).

3g: MS: m/z 566 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.71-3.20 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.73 (s, 6H, 2×-OCH_3), 6.80-8.00 (m, 10H, Ar-H).

3h: MS: m/z 566 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.71-3.20 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.73 (s, 6H, 2×-OCH_3), 6.80-8.00 (m, 10H, Ar-H).

3i: MS: m/z 611 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.71-3.20 (m, 8H, 4×-CH_2), 4.50 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.73 (s, 6H, 2×-OCH_3), 6.80-8.00 (m, 10H, Ar-H).

3j: MS: m/z: 562 (M^+); ^{1}H NMR (DMSO-d_6): δ 2.70-3.20 (m, 8H, 4×-CH_2), 4.53 (s, 1H, C=CH), 3.62 (s, 6H, 2×-OCH_3), 3.75 (s, 6H, 2×-OCH_3), 7.00-8.2 (m, 15H, Ar-H).

Antimicrobial Activity

The synthesized compounds have been subjected to various biological screening programmes.

The various screening programmes carried out include the *in vitro* study against gram-positive and gram-negative bacteria *viz*. *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and also *in vitro* antiviral study against human immunodeficiency virus (HIV) using different cell lines like *IIIB=HIV-1* and *ROD-HIV-2*. The antibacterial activity was determined using cup-plate agar diffusion method[21,22] by measuring the inhibition zones in mm. Evaluation of the antiviral activity of the compounds against HIV-1 strain IIIB and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described[23]. The MTT assay is based on the reduction of yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. Untreated control HIV- and mocke-infected cell samples were included for each sample.

For antibacterial activity, media, organism and solvent controls have been used for comparison. For anti-HIV activity MTT assay[25] control has been used. DMF was used as the solvent for solubilization.

SAR Studies

Ten novel compounds were synthesized in the present study and all were subjected to screening against various types of microorganisms.

2-Thiohydantoin and its arylidene derivatives showed excellent activity against *E. coli*, *S. aureus*, *S. typhi* and *B. subtilis*.

Antibacterial Activity

Styrylation of 2-thiohydantoins with their parent compound resulted in excellent antibacterial activity. Styrylation increases the activity as compared to the parent compounds. Compound **3b** showed maximum zone of inhibition (14 mm, 17 mm, 20 mm and 14 mm) against *E. coli*, *S. aureus*, *S. typhi* and *B. subtilis* (**Table II**). Structure activity relationship (SAR) studies reveal that halogens (Cl, Br, F) on *ortho*, *meta* and *para* positions within the phenyl ring shows

Table II—Antibacterial activity of compounds **3a-o**

Compd.	R	Antibacterial activity (Zone of inhibition in mm) at 50μg/mL concentration			
		E.coli	*S.aureus*	*S.typhi*	*B.subtilis*
3a	H	12	16	15	10
3b	3,4,5-$(OCH_3)_3$	14	17	20	14
3c	4-$N(CH_3)_2$	10	11	---	11
3d	4-F	13	15	18	---
3e	2-C_4H_3O	10	09	10	09
3f	4-OCH_3	13	15	14	11
3g	2-Cl	10	--	12	09
3h	3-Cl	12	11	13	10
3i	3-Br	10	10	16	15
3j	3-OC_6H_5	--	09	09	--
Tetracycline		15	19	24	21
Chloramphenicol		18	25	24	20

activity against all the microorganisms. Furthermore the studies showed that the methoxy group substitution resulted in greater inhibition as compared to the phenoxy group substitution.

Anti-HIV Activity

Ten thiohydantoin derivatives were prepared in this series and evaluated for their anti-HIV activity against IIIB (HIV-1) and ROD (HIV-2) cell cultures. All the data were calculated using the median OD (optical density) value of tree wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration for achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}). Results of the antiviral screening revealed that compounds **3a** and **3e** were not active while other compounds exhibited poor activity against both, HIV-1 and HIV-2 viruses. On the basis of SAR studies, it can be concluded that compounds of this series are not active or poorly active because they do not fit into the RT enzyme active site as known in the case of thiourea derivatives. They are well known for anti-HIV activity due to the action of physical forces like hydrogen bonding, volume fit groups, *etc*. The antiviral screening (HIV-1 and HIV-2) results of the novel synthesized compounds are tabulated in **Table III**.

Conclusions

It can be concluded that stryrylation of 2-thiohydantoin resulted in the following consequences:

(i) Biological screening results showed that styrylation with different aromatic aldehydes resulted in increase in antibacterial activity particularly when electron donating 3,4,5-trimethoxy group was present on phenyl ring attached to the methine linkage on the parent 2-thiohydantoins.

(ii) In case of antiviral activity, no improvement of HIV-1 and HIV-2 activity could be observed as compared to the parent compounds because of blocking of bisthiourea derivatives responsible for binding to the active site of RT enzyme *via* hydrogen bonding and other physical forces.

So it can be concluded that cyclization and styrylation of bisthiourea to obtain N,N′-bis(3,4-dimethoxyphenyl

Table III — Anti-HIV activity of compounds **3a-j**

Compd	Strain	Exp-nr	IC_{50} [a] (μg/mL)	CC_{50} [b] (μg/mL)	SI[c]	Max Prot	Appr.	av. IC_{50} (μg/mL)	SD	av. CC_{50} (μg/mL)	SD	SI
3a	IIIB	P3.3723	> 58.2	= 58.2	< 1	17	1					
		P3.3728	> 67.7	= 67.7	< 1	7	1	not active		60.83	6.00	<1
	ROD	P3.3729	> 56.6	= 56.6	< 1	6	2	not active		60.83	6.00	<1
3b	IIIB	P3.3723	> 14.3	= 14.3	< 1	18	1					
		P3.3728	> 12.6	= 12.6	< 1	16	1	>13.53		13.53	0.86	<1
	ROD	P3.3729	> 13.7	= 13.7	< 1	8	1	>13.53		13.53	0.86	<1
3c	IIIB	P3.3723	> 17.6	= 17.6	< 1	15	1					
		P3.3728	> 17.8	= 17.8	< 1	14	1	>18.17		18.17	0.81	<1
	ROD	P3.3729	> 19.1	= 19.1	< 1	13	1	>18.17		18.17	0.81	<1
3d	IIIB	P3.3723	> 18.9	= 18.9	< 1	19	1					
		P3.3728	> 18.1	= 18.1	< 1	12	1	>18.33		18.33	0.49	<1
	ROD	P3.3729	> 18	= 18	< 1	15	1	>18.33		18.33	0.49	<1
3e	IIIB	P3.3723	> 17.7	= 17.7	< 1	19	1					
		P3.3728	> 17	= 17	< 1	7	1	not active		17.33	0.35	<1
	ROD	P3.3729	> 17.3	= 17.3	< 1	1	1	not active		17.33	0.35	<1
3f	IIIB	P3.3723	> 84.7	= 84.7	< 1	11	1					
		P3.3728	> 72.8	= 72.8	< 1	18	1	>79.93		79.93	6.29	
	ROD	P3.3729	> 82.3	= 82.3	< 1	25	1	>79.93		79.93	6.29	
3g	IIIB	P3.3723	> 28.2	= 28.2	< 1	40	1					
		P3.3728	> 18	= 18	< 1	17	1	>21.90		21.90	5.51	<1
	ROD	P3.3729	> 19.5	= 19.5	< 1	3	1	>21.90		21.90	5.51	<1

—Contd

Table III — Anti-HIV activity of compounds **3a-j**—*Contd*

Compd	Strain	Exp-nr	IC_{50} [a] (µg/mL)	CC_{50} [b] (µg/mL)	SI[c]	Max Prot	Appr.	av. IC_{50} (µg/mL)	SD	av. CC_{50} (µg/mL)	SD	SI
3h	IIIB	P3.3723	= 23.9	= 67.5	= 3	51	1					
		P3.3728	> 52.4	= 52.4	< 1	45	1	> or =23.90		55.70	10.54	< or =2
	ROD	P3.3729	> 47.2	= 47.2	< 1	12	1			55.70	10.54	<1
3i	IIIB	P3.3723	> 125	> 125	X 1	5	1					
		P3.3728	> 93.4	= 93.4	< 1	10	1	>93.4		> or = 93.4		
	ROD	P3.3729	> 108	= 108	< 1	14	1	>108				
3j	IIIB	P3.3723	> 52	= 52	< 1	32	1					
		P3.3728	> 23.6	= 23.6	< 1	21	1	>33.5		33.50	16.03	<1
	ROD	P3.3729	> 24.9	= 24.9	< 1	22	1	>24.9		33.50	16.03	<1

a: IC_{50}- Inhibitory Concentration; b: CC_{50}- Cytotoxic Concentration of compound that reduces the viability of mock infected cell by 50% as determined by the MTT method[23]; c: Selectivity Index i.e. CC_{50}/IC_{50} ratio.

ethyl)-5-(arylidene)-2-thiohydantoins enhances antibacterial activity but not antiviral activity.

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