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ORIGINAL ARTICLE **SCIENCE & ENGINEERING**

SYNTHESIS AND CHARACTERIZATION OF SOME NOVEL HETERONUCLEAR *BIS*-THIAZOLIDINONE DERIVATIVES AND EVALUATION OF ITS ANTIMICROBIAL AND IN VITRO CYTOTOXIC PROPERTY

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Abstract

A series of novel *bis*-thiazolidinone derivatives 3(a-j) have been synthesized by the cyclization of thiosemicarbazones 2(a-j) with chloroacetic acid and sodium acetate. The integrated heterocyclic compounds were featured by chemical and spectroscopic methods such as IR, ^{1}H NMR and ^{13}C NMR. All the synthesized compounds have been screened for their antimicrobial activity against Gram-positive and Gram-negative bacteria such as *Staphylococcus Aureus, Bacillus licheniformis, Klebsiella pneumoniae, Escherichia coli* and antifungal activity against *Aspergillus niger* and in vitro cytotoxic activity against human cancer cell line (HeLa cell) and Vero cell line, using MTT assays but showed no activity.

Keywords: Thiosemicarbazone, 1,3-Thiazoldinone, antimicrobial & antifungal activity, cytotoxic activity.

Introduction

Heterocyclic chemistry is one of the core branches of the chemistry which constitutes a significant amount of research is currently going on it throughout the world. The study on heterocyclic systems draw interest both from the theoretical and pragmatic point of view. Design and development of new physiologically and pharmacologically active compounds heavily relies on heterocyclic chemistry. Heterocyclic compounds contain atom other than carbon in their ring have long been proven to have vivid biological activities (Zayda *et al*., 2020). The thiazolidinone class show strong similarity in characteristics with thiazolidine heterocyclic compounds and have various biological importance (Zhao *et al*.,2013). Thiazolidinone contains sulfur atom and nitrogen atom at position 1, and position 3, respectively; position 2, 4, or 5 is held by carbonyl group. Moreover, various other derivatives like 2-Thiazolidinone, 4-thiazolidinone, 5-thiazolidinone, 2-thioxo-4-thiazolidinone, thiazolidine-2, 4-dione are associated with a vast number of pharmacological properties (Rao *et al.,* 2011; Ismail *et al*., 2012; Chavan *et al*., 2010; Kumar *et al*., 2013; Zargi *et al.,*2008).

The 2-thiazolidinones have been recently examined as BRD4 bromodomain inhibitors (Zhao *et al*., 2013). Thus, thiazolidinone moiety is a magic part (wonder nucleus), which has been known to possess a wide

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spectrum of biological activities, such as antimicrobial (Srivastava *et al.,* 2002), anti-inflammatory (Ravindra *et al.,* 2020), antitubercular (Parekh *et al*., 2004) antidiabetic (Sameeh *et al*., 2022) and antiviral (Nitsche *et al.,* (2013).

Based on the above mentioned literature review and in continuation of our studies on the synthesis of heterocyclic compounds displaying biological activity, we observed in this study a successful reaction of thiosemicarbazide with dione derivatives to afford the corresponding thiosemicarbazones derivatives **2(a-j)** and subsequent cyclization with sodium acetate and chloroacetic acid gave novel heterocyclic compounds bearing double fold thiazolidinone functionality such as *bis*-thiazolidinone derivatives **3(a-j)** which might have potential antimicrobial and cytotoxic activities. But the investigation showed no significant antibacterial, antifungal and cytotoxic properties.

Materials and Methods

General

An open capillary tube was used to estimate the melting points accurately. Through thin layer chromatography, homogeneity of the compounds was determined. The IR spectrum of the compounds were taken on Schimadzo-FTIR infrared spectrometer. The ^{1}H NMR & ^{13}C NMR spectra were recorded on a varian in $CDCl_3$ and DMSO-d_6.

Chemistry

General procedure for the synthesis of bis-thiazolidinone derivatives 3(a-j)

The *bis*-thiazolidinone derivatives 3(a-j) were synthesized by refluxing the mixture of *bis*- thiosemicarbazone derivatves 2(a-j) in presence of chloroacetic acid and fused sodium acetate in ethanol for 4-6 hours with continuous stirring. The progress of the reaction was monitored by TLC. Then the reaction mixtures were turned into cool at room temperature and poured into ice water. The separated solids were purified by recrystallization from ethanol.

Table 1. *Bis*-thiazolidinone compounds 3a-3j

Compound No.	Compound name	Yield %	m.p ^{o}C
3a	*(2,2')-2,2'-(((1,3)-5,5-dimethyl-2-(2-(2-nitrophenyl) hydrazineylidene) cyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis (thiazolidin-4- one) 3a*	67	210-215
3b	*(2,2')-2,2'-(((1,3)-2-(2-(4-chlorophenyl) hydrazono)-5,5-Dimethylcyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3b*	55	220-225
3c	*(2,2')-2,2'-(((1,3)-5,5-dimethyl-2-(2-(4-nitrophenyl) hydrazono) cyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one)) 3c*	65	220-225
3d	*(2,2')-2,2'-(((1,3)-2-(2-(4-bromophenyl) hydrazono)-5,5-Dimethylcyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3d*	45	330-335
3e	*(2,2')-2,2'-(((1,3)-5,5-dimethyl-2-(2-(p-tolyl) hydrazono) cyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3e*	50	205-210

3f	*(2,2')-2,2'-(((1,3)-5,5-dimethyl-2-(2-(o-tolyl) hydrazono) cyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3f*	60	210-215
3g	*(2,2')-2,2'-(((1,3)-2-(2-(2-methoxyphenyl) hydrazono)-5,5-dimethylcyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3g*	60	225-230
3h	*(2,2')-2,2'-(((1,3)-2-(2-(4-methoxyphenyl) hydrazono)-5,5-dimethylcyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3h*	48	230-235
3i	*(2,2')-2,2'-(((1,3)-5,5-dimethyl-2-(2-phenylhydrazono) cyclohexane-1,3-diylidene) bis (hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3i*	49	215-220
3j	*(2,2')-2,2'-(((1,3)-2-(2-(4-acetylphenyl) hydrazono)-5,5-dimethylcyclohexane-1,3-diylidene) bis(hydrazine-2,1-diylidene)) bis(thiazolidin-4-one) 3j*	58	220-225

Table 2. FT-IR (KBr, ν_{max} /cm^{-1}) 3a-3j

Comp. No.	N-H	C=H (Ar)	C=H(Alph.)	C=O	C=N	C=C (Ar)	C-X X= Cl, Br
3a	3442.086 & 3349.50	3254.02	2961.79, 2861.46	1652.52	1605.79	1478.84	
3b	3442.09	3054.38	2954.079, 2876.92	1675.23	1620.25	1416.70, 1390.72	830.38
3c	3372.64, 3265.69	3179.75	2966.61	1645.33	1625.08	1495.84	
3d	3451.73	3053.41	2956.91, 2931.89	1676.91	1619.22	1583.61, 1506.65, 1417.72	829.41
3e	3464.26, 3304.17	3169.14	2953.11	1650.00	1608.68	1489.095	
3f	3213.51 & 3208.04	3146.96	2956.00, 2873.06	1650.51	1638.23	1490.33, 1400.00	
3g	3229.26	3154.68	2957.93	1716.70, 1655.69	1598.08	1506.35, 1418.37	
3h	3425.69	3155.64	2955.04	1634.72	1596.82	1511.27, 1498.99	
3i	3222.19	3150.82	2956.97	1664.64	1596.14	1493.91	
3j	3434.37	3185.54	2956.00	1667.6	1623.15	1514.17, 1393.61	

Table 3. ^{1}H NMR ($CDCl_3$) δ ppm 3a-3j

Comp. No.	CH_3	CH_2	Active Methylene	Ar-H	N-H	OCH_3, $COCH_3$, Ar-CH_3
3a	1.125 (S, 6H)	1.258 (S, 4H)	2.598 (S, 4H)	7.265 (t, 1H, J = 15.2), 7.816 (t, 1H, J = 15.6), 8.288 (d, 2H, J = 8.4),	12.936 (S, 1H), 15.856 (S, 1H).	
3b	1.139 (S, 6H)	1.640 (S, br, 2H), 2.485(S, 2H),	2.633 (S, 4H)	7.391(d, 2H, J=8.8), 7.505(d, 2H, J=8.8).		
3d	1.156 (S, 6H)	1.596 (S, 4H)	2.633(S, 4H)	7.45(d, 2H, J=8.8), 7.55(d, 1H, J=8.8).		
3f	1.107 (S, 6H)	1.414 (S, 4H)	2.518(S, 4H)	6.329 (S, 1H), 7.128 (d,1H, J=8), 7.16(d, 1H, J=8), 7.42 (d, 1H, J=8), 8.016 (d, 1H, J=8)	12.994 (S, 1H)	2.567 (S, 3H, Ar-CH_3)

Table 4. ^{1}H NMR (DMSO-d_6) δ ppm 3a-3j

Comp. No.	CH_3	CH_2	Active Methylene	Ar-H	N-H	
3c	1.041(S, 6H)	2.633 (S, 4H)	2.633 (S, 4H)	7.633 (S, 1H), 8.066 (S, 1H), 8.22 (d, 2H, J=8.8 Hz),	11.095 (S, 1H), 13.116 (S, 1H).	
3e	0.966 (S, 3H), 1.021 (S, 3H)	2.269 (bd, S, 2H), 2.286 (S, 2H)	2.702(S, 2H), 2.772 (S, 2H),	7.160 (m, 1H), 7.382 (m, 1H), 7.684 (bd, S, 1H), 8.390 (bd, S, 1H)	13.023 (S, 1H), 13.156 (S, 1H), 14.259 (S, 1H).	3.644 (S, 3H, Ar-CH_3)
3g	1.019 (S, 6H)	2.537 (bd, S, 4H)	2.566 (S, 2H), 3.850 (S, 2H)	7.030 (bd, S, 1H), 7.186 (bd, S, 1H), 7.951 (bd, S, 1H), 8.504 (S, 1H)	12.608 (S, 1H), 14.843 (S, 1H)	3.953 (S, 3H, Ar-OCH_3)
3h	0.966 (S, 3H), 1.021 (S, 3H)	2.268 (S, 2H), 2.286 (S, 2H)	2.432 (S, 2H), 2.702 (S, 2H)	7.509 (d, 1H, J=8.8), 7.662 (d, 1H, J=8.0), 7.9140 (d, 1H, J=8.0), 8.420 (d, 1H, J=8.0)	13.096 (S, 1H), 13.156 (S, 1H), 14.259 (S, 1H)	4.080(S, 3H, OCH3)
3i	1.033 (S, 6H)	2.447 (S, 2H), 4.093 (S, 2H)	2.623 (S, 4H)	7.171 (t, 1H, J=14), 7.387 (d, 1H, J=6.8), 7.590 (d, 1H, J=8), 7.646 (d, 1H, J=7.6), 8.450 (d, 1H, J=12)	12.509 (S, 1H), 13.00(S, 1H), 14.292(S, 1H).	
3j	0.981 (S, 3H), 1.003 (S, 3H)	1.912 (S, 2H)	2.168 (S, 2H), 2.177 (S, 2H)	7.418 (d, 1H, J=8.0), 7.495 (d, 1H, J=8.2), 7.862 (d, 1H, J = 8.4), 7.901 (d, 1H, J = 8.1)	14.133 (S, 1H) 14.609 (S, 1H).	2.951 (S, 3H, OCH_3)

Table 5. ^{13}CNMR ($CDCl_3$) δ ppm 3a-3j

Comp. No.	C=O	C=N moiety	Aromatic-C	tert-C	CH_2	CH_3
3a	197.645 (1C), 179.390(1C)	138.366 (2C), 137.523 (1C)	136.836 (C-NH), 135.595, 132.368, 126.264, 124.434, 118.110 (5C)	58.488 (1C), 52.916 (1C, =C=),	47.086 (2C), 30.736 (2C)	27.993 (2C)
3d	197.389 (2C), 193.245 (2C)	140.189 (1C), 130.52 (1C)	132.743 (2C), 120.26(1C), 118.857 (3C)	52.629 (1C)	52.602 (2C) 30.736 (2C),	28.516 (2C)
3f	197.931(2C), 179.125 (1C)	139.193(2C), 138.945 (1C)	130.948(1C), 129.594 (1C), 128.393(1C), 126.083(1C), 125.415 (1C), 115.801 (1C)	47.114 (1C)	30.706 (2C), 27.954 (2C)	55.454 (1C, Ar-CH_3),17.137(2C)

Table 6. ^{13}C NMR (DMSO-d6) δ ppm 3a-3j

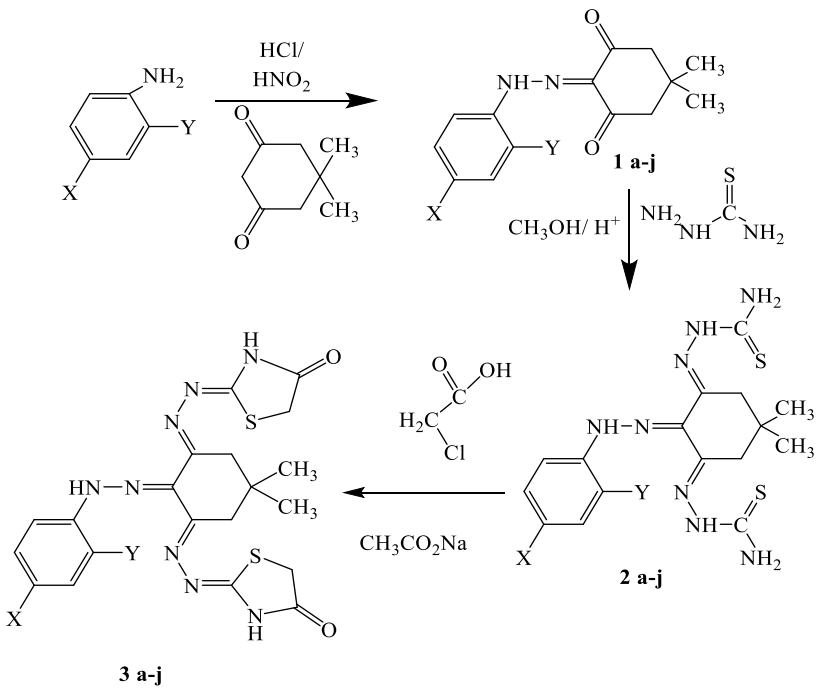
Comp. No.	C=O	C=N moiety	Aromatic-C	tert-C	CH_2	CH_3
3e	178.535 (1C), 174.095 (1C)	165.387(1C), 162.357(1C), 141.049(1C), 140.357 (1C)	138.947 (1C), 133.645 (1C), 132.855 (1C), 130.473 (1C), 130.320 (1C), 128.571 (1C)	46.795 (1C)	46.546 (1C), 34.530(1C), 30019 (1C), 28.872 (1C)	28.226 (1C), 20.852 (1C)
3g	198.575 (2C),	178.504 (1C), 148.249 (1C), 138.683 (1C)	130.515 (1C), 130.280 (1C), 126.421 (1C), 122.066 (1C), 115.162 (1C), 112.436 (1C)	30.739 (1C)	52.264 (2C), 46.479 (2C),	56.744 (1C, OCH_3), 28.004 (2C)
3h	181.910 (2C), 178.505 (2C)	165.387 (2C), 162.357 (3C),	141.049 (1C), 133.645 (1C), 130.653 (1C), 128.751 (1C), 116.174 (1C)	20.152 (1C)	40.200 1C), 34.530 (1C), 30.019 (1C)	46.795 (1C, OCH_3), 28.821 (2C)
3i	198.477 (2C), 192.915 (2C)	178.593 (1C), 174.195 (1C), 162.385 (1C)	142.368 (1C), 141.524 (1C), 138.814 (1C), 138.679 (1C), 130.22 (1C), 129.410 (1C)	52.313 (1C)	46.762 (1C), 46.516 (1C), 30.735 (1C), 29.891 (1C)	28.318 (1C), 27.991 (1C)

Results and Discussion

Chemistry

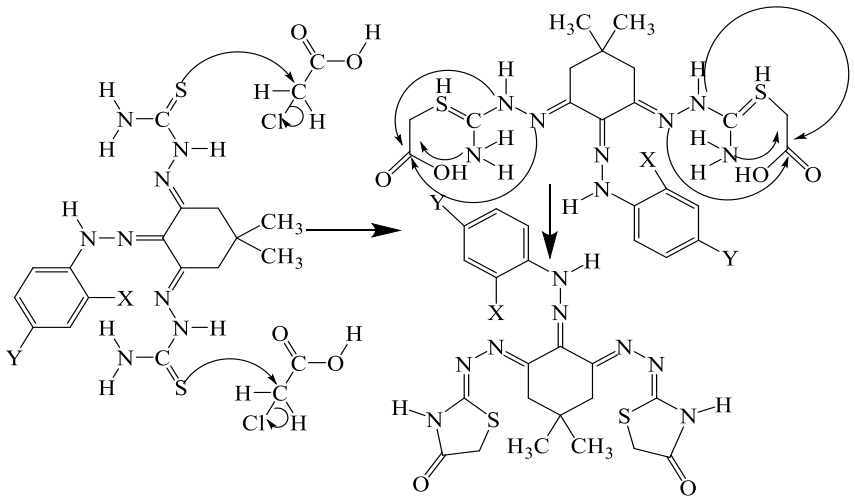
The synthetic procedure adopted to obtain the target compounds are outlined in the scheme 1.

The key intermediate thiosemicarbazone derivatives 2a-j were prepared by the reaction of 2-arylhydrazido-hexane-1,3-dione derivatives 1a-j with thiosemicarbazide. Refluxing thiosemicarbazone derivatives 2(a-j) with chloroacetic acid in presence of anhydrous sodium acetate in ethanol (Prakasha *et al.,* 2011) afforded 1,3-thiazolidinone derivatives 3(a-j). The IR spectrum of 3 showed a new band at 1650 cm^{-1}



Here X= H, Br, Cl, NO_2, CH_3, OCH_3, $COCH_3$
& Y= H, CH_3, OCH_3

Scheme 1



Scheme 2

Figure 1. Plausible mechanism for the Thiazolidinone ring formation.

attributed to a long-conjugated C=O group of thiazolidinone ring. The ^{1}H NMR spectrum of 3 showed a new signal at δ 2.6 ppm attributed to the CH_2 proton of thiazolidinone ring. The downfielded N-H protons were found at 12-14 ppm. The ^{13}C NMR of 3 showed the signals at ∂ 178, 174, 165, 162, 128, 30.01, 24.87 and 20. 85 ppm for C=O, C=O, C=N, C-N, C=C, CH, CH_2 and CH_3 respectively. The compounds 3 showed no antimicrobial and cytotoxic property because the hetero ring of two opposite sides counter act each other. In one side the hetero ring binds as active site but opposite ring interacts by van der Waal's forces.

Antimicrobial activity

Compounds 3a, 3c, 3d, 3e, 3h, 3i and 3j were evaluated against gram-positive, gram-negative bacteria and fungi strains. The Gram Positive as well as Gram Negative bacteria used in the present investigation was found to be completely resistant all thizolidinone compounds at a dose of 30μg/ml disc. And the fungal used in the present investigation was found to be completely resistant all thoazolidinone compounds at a dose of 30μg/ml disc.

In vitro cytotoxicity screening

The newly synthesized compounds 3a, 3d and 3h were evaluated for their in vitro cytotoxic effects against human cancer cell (HeLa) cell line. All Compounds were inactive cytotoxic agent against human cancer (HeLa) cell line.

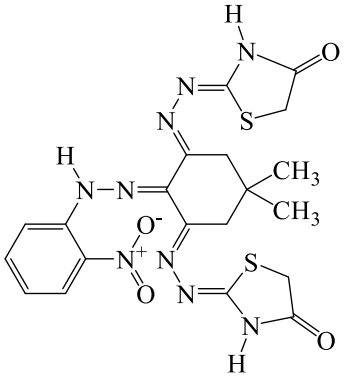
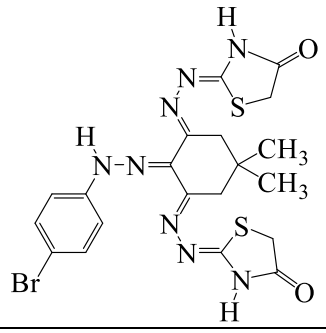
Table 7.

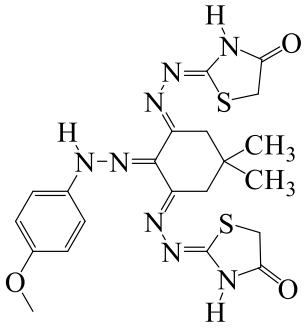
Diameter Of Zone of Inhibition (mm)				
Sample code	Microorganisms			
	Gram Positive		Gram Negative	
	Staphylococcus Aureus	*Bacillus licheniformis*	*Klebsiella pneumoniae*	*Escherichia coli*
Chloramphenicol (Standard)	23	17	23	25
3a	-	-	-	-
3c	-	-	-	-
3d	-	-	-	-
3e	-	-	-	-
3h	-	-	-	-
3i	-	-	-	-
3j	-	-	-	-

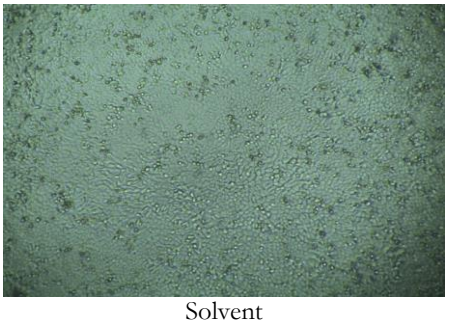
Table 8.

Diameter Of Zone of Inhibition (mm) (*Bis*- thiazolidinone compounds)	
Sample code	Fungus (Aspergillus Niger)
Amphotericin B (Standard)	10
3a	-
3c	-
3d	-
3e	-
3h	-
3i	-
3j	-

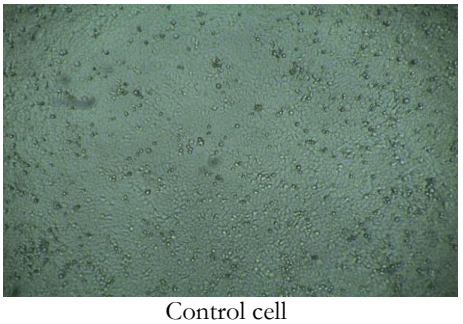
Table 9.

Sample code	Sample/Compound's structure	Survival of cells (HeLa)	Remarks
Solvent		100%	observed on inactive HeLa cell line
Control cell		>95%	
3a		>95%	
3d		>95%	

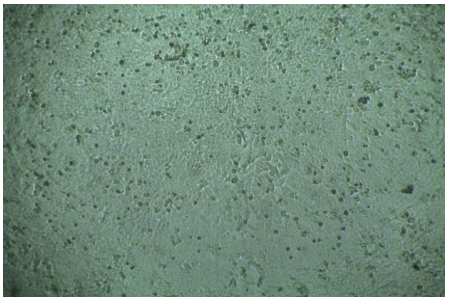
3h		>95%



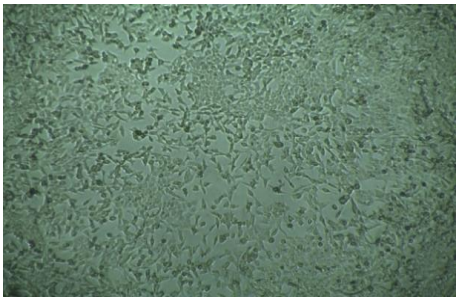
Solvent



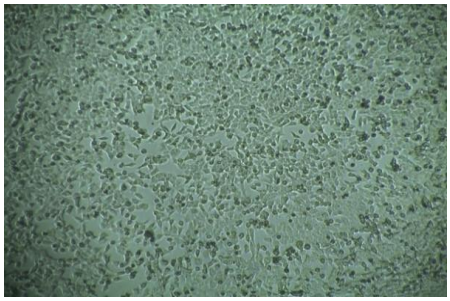
Control cell



3a



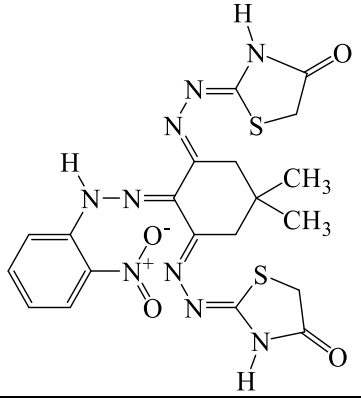
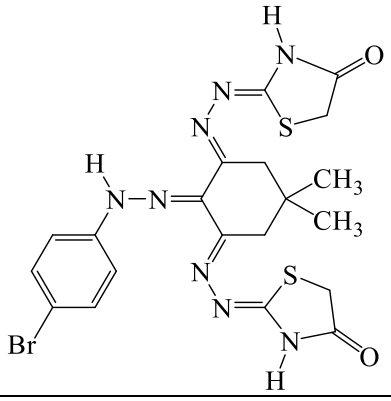
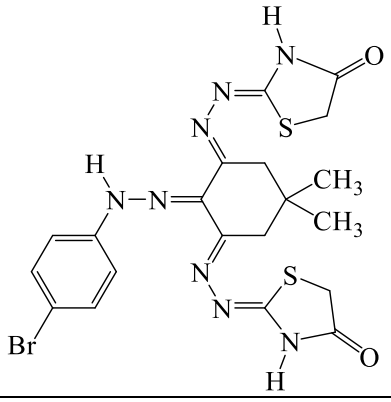
3d

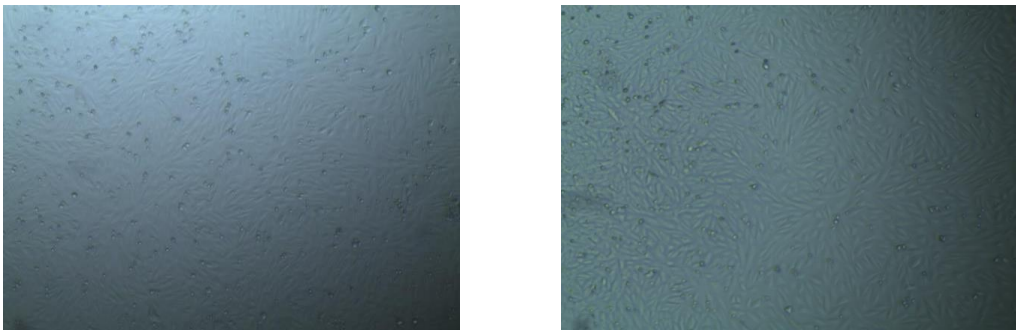


3h

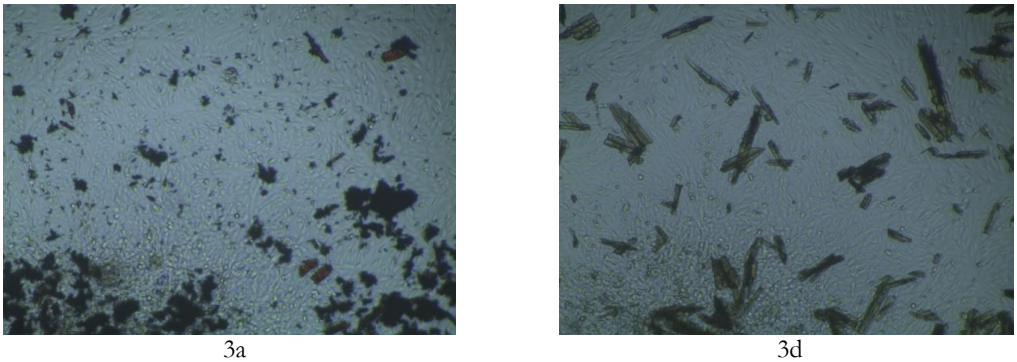
Figure 2. Pictorial view change in HeLa cell.

Table 10.

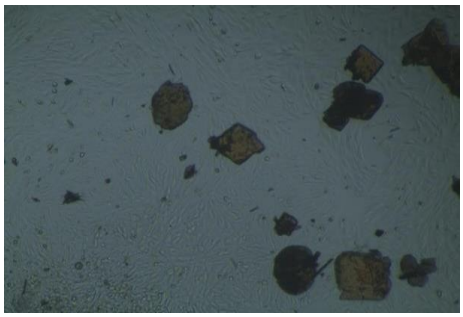
Sample code	Sample / Compound's structure	(Vero)	Remarks
Solvent		100%	
Control cell		>95%	
3a		>95%	
3d		>95%	No Cytotoxicity was observed on Vero cell line
3h		>95%	



Solvent Control cell



3a 3d



3h

Figure 3. Pictorial view change in Vero cell.

Conclusions

In conclusion, a series of novel heterocyclic compounds *bis*-thiazolidinone derivatives were synthesized in moderate to good yield derived from different thiosemicarbazones and they were characterized by different spectroscopic methods such as IR, ^{1}H NMR and 13C NMR. All the synthesized compounds have been screened for their antimicrobial activity against Gram-positive and Gram-negative bacteria such as *Staphylococcus Aureus, Bacillus licheniformis, Klebsiella pneumoniae, Esherichia coli* and antifungal activity against *Aspergillus niger* and evaluated in vitro cytotoxic activity against human cancer cell line (HeLa cell), using MTT assays but no significant activity was found.

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Author disclosure statement

There is no conflict of interest among the authors

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