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Synthesis, DNA cleavage and antimicrobial activity of 4-thiazolidinones-benzothiazole conjugates

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Antimicrobial screening of several novel 4-thiazolidinones with benzothiazole moiety has been performed. These compounds were evaluated for antimicrobial activity against a panel of bacterial and fungal strains. The strains were treated with these benzothiazole derivatives at varying concentrations, and MIC's were calculated. Structures of these compounds have been determined by spectroscopic studies viz., FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Significant antimicrobial activity was observed for some members of the series, and compounds viz. 3-(4-(benzo[d]thiazol-2-yl) phenyl)-2-(4-methoxyphenyl)thiazolidin-4-one and 3-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-hydroxy phenyl)thiazolidin-4-one were found to be the most active against *E.coli* and *C.albicans* with MIC values in the range of 15.6–125 µg/ml. Preliminary study of the structure–activity relationship revealed that electron donating groups associated with thiazolidine bearing benzothiazole rings had a great effect on the antimicrobial activity of these compounds and contributes positively for the action. DNA cleavage experiments gave valuable hints with supporting evidence for describing the mechanism of action and hence showed a good correlation between their calculated MIC's and its lethality.

Keywords: Benzothiazoles, 4-thiazolidinones, DNA cleavage, MIC

In recent years, the number of life threatening infections caused by multiple drug resistant pathogens has reached to an alarming level in hospitals, community and have become a global public health problem. These resistance problems demands renewed efforts should be made for effective therapy.

Benzothiazole and its derivatives are widely distributed in nature and are well known to exhibit broad spectrum of biological activities such as antimicrobial[1], antiproliferative[2], antimalarial[3], anticonvulsant[4], antihelmintic[5], analgesic[6], anti-inflammatory[7] and antidiabetic[8]. Small ring heterocycles, particularly 4-thiazolidinones have shown important biological activities i.e anti-inflammatory[9], antitubercular[10], antimicrobial[11], antiviral[12] and anti-HIV[13]. Therefore, it was envisaged that chemical entities with benzothiazole and 4-thiazolidinone moieties would result in compounds of interesting biological activities. In view of these, an attempt was made to incorporate these biologically active components together to give a confined structure like the titled compounds and evaluate their antimicrobial activity.

Synthesis and biological properties of various Schiff base-benzothiazole hybrids have been reported[14]. These compounds were screened for their antibacterial and antifungal activities and it was found that some of them have moderate to good biological properties. The biological significance of this class of compounds impelled us to extend this series by working on the synthesis of new 4-thiazolidinone derivatives. In this communication synthesis of some new 4-thiazolidinone derivatives derived from Schiff bases of benzothiazoles and their antimicrobial studies have been reported.

Some of the metal free ligands have shown promising nucleolytic activity. The key functionality at the active site in natural nucleases *viz.* staphylococcal nuclease (SNase)[15] and bovine pancreatic ribonuclease (RNase A)[16], are the positive charge groups (guanidinium or ammonium). The binding ability of this positive group with the phosphate of DNA or RNA (negative group) through hydrogen bonding and electrostatic interaction[17] in biological molecules imparts cleavage of phosphodiester

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bond[18-22]. Some of the compounds with positive charge groups, as nuclease mimics for cleavage of phosphodiester bond, are identified as efficient cleavers of RNA[23]. Thus the compounds designed in such manner were artificial nuclease mimics.

Keeping the above information in view, the DNA cleavage activity of 4-thiazolidinones-benzothiazole conjugates has been studied by gel electrophoresis.

Materials and Methods

Chemistry—The target compounds 4-thiazolidinones-benzothiazole conjugates (TB01-10) were obtained by adopting the following steps: equimolar quantities of Schiff bases of benzothiazole hybrids reported earlier[14] and thioglycolic acid, were refluxed in suitable solvent (DMF) in presence of anhyd. $ZnCl_2$ as catalyst which yielded substituted 4-thiazolidindione benzothiazole derivatives. The IR, ^{1}H-NMR, ^{13}C-NMR spectral data of compounds (TB01-10) were in accordance with the proposed molecular structures.

Experimental—All the reactions were monitored with the help of thin-layer chromatography using pre-coated aluminium sheets with GF_{254} silica gel, 0.2 mm layer thickness (E. Merck). Melting points were determined in open-glass capillaries on Stuart-SMP10 melting point apparatus and were reported uncorrected. IR absorption spectra were recorded on Shimadzu FTIR-8400s. ^{1}H NMR spectra were recorded on the Bruker DRX-300 FTNMR and ^{13}C NMR spectra were recorded on the JEOL AL300 FTNMR spectrometer operating at 300 MHz. Chemical shifts were measured relative to internal standard TMS (δ:0) and were reported as parts per million (ppm) downfield from TMS. Elemental analyses (C, H, N) were performed on Exeter Analytical Inc., USA, CE-440 elemental analyzer.

General procedure for the synthesis of 4-thiazolidinones-Benzothiazole Conjugates from Schiff bases of benzothiazoles [3-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(substituted-phenyl)thiazolidin-4-one (TB01-10)]—To the equimolar solution of respective Schiff bases of benzothiazole (0.01 mol) in DMF and of thioglycolic acid solution (0.01 mol), certain amount of anhyd. $ZnCl_2$ was added as catalyst. The reaction mixture was refluxed on a water bath for 10-12 h. After cooling it to room temperature, the reaction mixture was poured on to the crushed ice, neutralized with K_2CO_3 to get the desired product. The solid that separated was filtered and dried. It was further recrystallised by ethanol/methanol.

Characterization data of the compounds are given in Table 1. Physicochemical data and results of elemental analysis of the compounds are listed in Tables 2.

Antimicrobial susceptibility testing

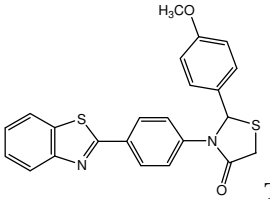
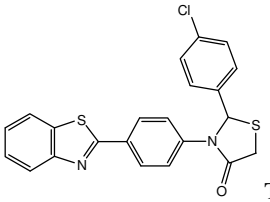
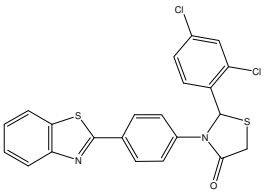
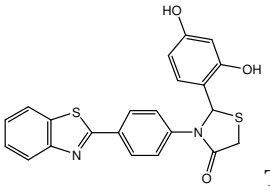
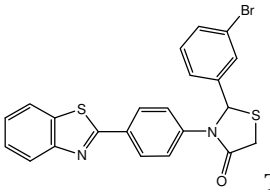
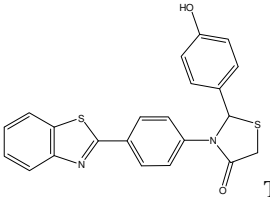
Microorganisms—The compounds (TB01-10) were screened for their antibacterial activity against various pathogenic bacterial strains (Gram-negative and Gram-positive) viz., *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25323), *Salmonella typhi* (MTCC 3216), *Pseudomonas aeruginosa* (ATCC 27893) and *Enterococcus faecalis* (Clinical isolate). Anti-fungal activity of the above compounds was evaluated against fungal strains viz. *Candida albicans* (ATCC 90028) and *Candida tropicalis* (ATCC 750).

Preparation of inoculum—The inoculum size of the test isolates was standardized according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997)[24]. The bacterial isolates were inoculated in Mueller–Hinton Agar (MHA, Hi-Media) and fungal isolates in Sabouraud dextrose agar (SDA, Hi-Media)[25]. The inoculums were incubated at 37 ºC for 3-6 h until the culture attained the turbidity to the Mc Farland Standard no: 0.5 [~ 10^6 colony forming units (CFU) per mL].

Disc Diffusion method—Susceptibility test was performed by agar disc diffusion method. Standard inoculum (~ 10^6 CFU/mL or 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The Whatman no. 1 filter paper discs (6 mm in diam.) impregnated with the test compounds (20 mL/disc) placed on the plates. Ciprofloxacin (5 mg/disc, Hi-Media) and fluconazole (10 mg/disc, Hi-Media) were used as positive controls for bacteria and fungi respectively. DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C and 48 h at 37 °C for bacteria and fungi respectively. The susceptibility was assessed on the basis of diameter of zone of inhibition measured in millimetres. All the tests were performed in triplicate and the average was taken as final reading.

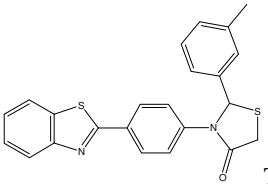
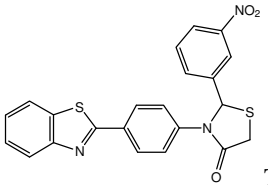
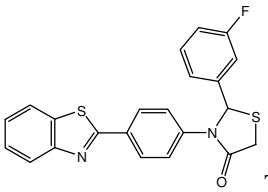
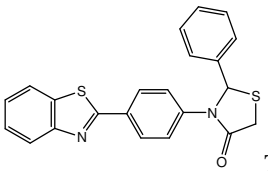
Determination of MIC—Solutions of the test compounds, ciprofloxacin and fluconazole were prepared in DMSO at a concentration of 500 µg/mL. From this stock solution, serial dilutions of the compounds (250, 125 ..... 3.91 µg/mL) were prepared to determine the minimum inhibitory

Table 1 — Spectral data of compounds (TB01-10)

S. No.	Compound Structure and code	IR Stretching in cm^{-1}	^{1}H NMR shift (δ) ppm	^{13}C NMR shift (δ) ppm
1	 TB01	1658.84 (C=O str. of carbonyl group), 2850 (C–H str., O–CH_3 group) 3221.23 (Aromatic –C–H str.)	3.72 (s, 3H, OCH_3), 4.81 (s, -CH of Thiazolidindione ring), 3.1 (s, 2H, -CH_2 of Thiazolidindione ring), 7.60–7.89 (m, 12H, Ar–H)	55.69 (OCH_3) , 65.70 (-CH of Thiazolidindione ring), 33.11 (-CH_2 of Thiazolidindione ring), 183.34 (C=O), 126.67–140.05 (Aromatic–C, C_2–C_9 $C_{1'}$–$C_{6'}$ $C_{1''}$–$C_{6''}$)
2	 TB02	1683.91 (C=O str. of carbonyl group), 1065 (C–Cl str.), 3131.23(Aromatic –C–H str.)	4.51 (s, -CH of Thiazolidindione ring), 3.20 (s, 2H, -CH_2 of Thiazolidindione ring), 7.69–7.90 (m, 12H, Ar–H)	67.71 (-CH of Thiazolidindione ring), 32.40 (-CH_2 of Thiazolidindione ring), 181.14 (C=O), 123.07–145.05 (Aromatic–C, C2–C9 C1'–C6' C1" –C6")
3	 TB03	1632.54 (C=O str. of carbonyl group) 1108, 1065 (C–Cl str.), 3118, 3083 (Aromatic C–H str.)	4.11 (s, -CH of Thiazolidindione ring), 3.20 (s, 2H, -CH_2 of Thiazolidindione ring), 7.42–7.91 (m, 11H, Ar–H)	63.51 (-CH of Thiazolidindione ring), 38.40 (-CH_2 of Thiazolidindione ring), 178.14 (C=O), 121.07 –142.05 (Aromatic–C, C2–C9 C1'–C6' C1" –C6")
4	 TB04	1628.34 (C=O str. of carbonyl group), 3126 (O–H str., br.), 3121.03(Aromatic –C–H str.)	10.69 (br, s, 1H, o–OH group), 9.92 (s, 1H, p–OH group), 4.30 (s, -CH of Thiazolidindione ring), 3.61 (s, 2H, -CH_2 of Thiazolidindione ring), 6.91–7.54 (m, 11H, Ar–H)	64.01 (-CH of Thiazolidindione ring), 35.31 (-CH_2 of Thiazolidindione ring), 174.14 (C=O), 122.07–146.05 (Aromatic–C, C2–C9 C1'–C6' C1"–C6")
5	 TB05	1641.13 (C=O str. of carbonyl group), 765 (C–Br str.), 3221.73(Aromatic –C–H str.)	4.21 (s, -CH of Thiazolidindione ring), 3.30 (s, 2H, -CH_2 of Thiazolidindione ring), 7.01–7.84 (m, 12H, Ar–H)	67.62 (-CH of Thiazolidindione ring), 32.11 (-CH_2 of Thiazolidindione ring), 179.24 (C=O), 125.07 –149.15 (Aromatic–C, C2–C9 C1'–C6' C1" –C6")
6	 TB06	1644.21 (C=O str. of carbonyl group), 3426 (O–H str.), 3271.61(Aromatic –C–H str.)	9.72 (s, 1H, –OH), 4.41 (s, -CH of Thiazolidindione ring), 3.40 (s, 2H, -CH_2 of Thiazolidindione ring), 7.11–7.94 (m, 12H, Ar–H)	65.21 (-CH of Thiazolidindione ring), 34.50 (-CH_2 of Thiazolidindione ring), 182.84 (C=O), 122.37–150.15 (Aromatic–C, C2–C9 C1'–C6' C1"–C6")

…Contd

Table 1 —Spectral data of compounds (TB01-10)…*Contd*

S. No.	Compound Structure and code	IR Stretching in cm^{-1}	^{1}H NMR shift (δ) ppm	^{13}C NMR shift (δ) ppm
7	 TB07	1638.13 (C=O str.of carbonyl group), 2892 (C–H str., $–CH_3$ group), 3171.61(Aromatic –C–H str.)	2.82 (s, 3H, $–CH_3$), 4.12 (s, -CH of Thiazolidindione ring), 3.4 (s, 2H, $-CH_2$ of Thiazolidindione ring), 7.10–7.89 (m, 12H, Ar–H)	62.21 (-CH of Thiazolidindione ring), 31.81 ($-CH_2$ of Thiazolidindione ring), 180.14 (C=O), 124.37 –152.15 (Aromatic–C, C2–C9 C1'–C6' C1"–C6")
8	 TB08	1624.22 (C=O str. of carbonyl group), 1351 (N=O str.), 3154.03(Aromatic –C–H str.)	4.61 (s, -CH of Thiazolidindione ring), 3.30 (s, 2H, $-CH_2$ of Thiazolidindione ring), 7.06–7.90 (m, 12H, Ar–H)	64.30 (-CH of Thiazolidindione ring), 34.51 ($-CH_2$ of Thiazolidindione ring), 182.44 (C=O), 121.47–155.25 (Aromatic–C, C2–C9 C1'–C6' C1"–C6")
9	 TB09	1638.23 (C=O str. of carbonyl group), 3254.87 (Aromatic –C–H str.)	4.50 (s, -CH of Thiazolidindione ring), 3.60 (s, 2H, $-CH_2$ of Thiazolidindione ring), 7.10–7.68 (m, 12H, Ar–H)	62.31 (-CH of Thiazolidindione ring), 36.90 ($-CH_2$ of Thiazolidindione ring), 185.40 (C=O), 123.07 –153.85 (Aromatic–C, C2–C9 C1'–C6' C1" –C6")
10	 TB10	1640.53 (C=O str. of carbonyl group), 3284.03(Aromatic –C–H str.)	4.11 (s, -CH of Thiazolidindione ring), 3.30 (s, 2H, $-CH_2$ of Thiazolidindione ring), 7.12–7.99 (m, 13H, Ar–H)	61.70 (-CH of Thiazolidindione ring), 34.91 ($-CH_2$ of Thiazolidindione ring), 178.54 (C=O), 120.67 –145.05 (Aromatic–C, C2–C9 C1'–C6' C1" –C6")

concentration (MIC). All of the dilutions were made with distilled water. At the end of the incubation period, the MIC values were determined. All determinations were done in triplicates and the average was taken as final reading. The standard antibiotic, ciprofloxacin (500 μg/mL) for bacteria and fluconazole (500 μg/mL) for fungi were used as positive controls and 500 μL of DMSO used as a negative control. The lowest concentration of the compound that prevented visible growth (turbidity on liquid media) was considered as MIC.

Nuclease activity

Cleavage experiments were performed by agarose gel electrophoresis. The illuminated gel was photographed by Alpha Innotech Corporation Instrument. Cleavage experiments of plasmid DNA (200 ng) by TB01, TB06, TB08 and TB09 (10-30 μM) in (5 mM Tris-HCl/50 mM NaCl), buffer (*p*H 7.2) were carried out. The samples were incubated for 1 h at 37 °C. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol was added and electrophoresis was carried out at 60 V for 1 h in Tris-HCl buffer using 1% agarose gel containing 1.0 μg/mL ethidium bromide (EB)[26]. The reaction was also monitored upon addition of various radical inhibitors and/or activators such as DMSO, tert-butyl alcohol (TBA) and sodium azide (NaN_3).

Results and Discussion

The structures of 4-thiazolidinones-benzothiazole conjugates (TB01-10) were confirmed by their spectral analysis. The FT-IR spectra showed absorption bands at 3,083–3,284 cm^{-1} for aromatic C–H and at 1,624-1,683 for C=O str. of carbonyl

group. The ^{1}H NMR showed sharp singlet peak in the range of δ 4.11–4.81 ppm indicating the presence of -CH of thiazolidindione ring. The singlet peak in the range of δ 3.10-3.61 ppm indicated the presence -CH_2 of thiazolidindione ring. The multiplet at δ 6.91–7.99 ppm was due to aromatic protons. Moreover, ^{13}C NMR spectra revealed all the corresponding peaks in the range of δ 61.70–67.71 ppm and δ 31.81–38.40 ppm which were due to -CH of thiazolidindione ring and -CH_2 of thiazolidindione ring respectively. The peaks appearing in the range of δ 174.14–185.40 ppm corresponds to C=O of the thiazolidinone ring and of δ 120.67–155.25 ppm corresponds to aryl carbon.

The conjugates 4-thiazolidinone benzothiazoles were screened *in vitro* for antibacterial and antifungal activity against five bacterial and two fungal remarkable strains. Physicochemical data and results of elemental analysis of the compounds are listed in Table 2. The results of anti-microbial studies (inhibition zone of diameter and MIC) of all the novel compounds are presented in Table 3. Significant antimicrobial activity was observed for some of the

Table 2 — Analytical and physicochemical data of the synthesized compounds

Compound code	Molecular formula	M.W.[a]	M.p.(°C)[b]	Yield (%)	% Analysis of C, H,N found (calc.)[c]		
					C	H	N
TB01	$C_{23}H_{18}N_2O_2S_2$	418.53	245-247	54	66.01(66.00)	4.28(4.33)	6.65(6.69)
TB02	$C_{22}H_{15}ClN_2OS_2$	422.95	261-263	62	62.45(62.47)	3.54(3.57)	6.62(6.62)
TB03	$C_{22}H_{14}Cl_2N_2OS_2$	457.40	255-257	56	57.75(57.77)	3.06(3.09)	6.10(6.12)
TB04	$C_{22}H_{16}N_2O_3S_2$	420.50	224-226	68	62.83(62.84)	3.82(3.84)	6.63(6.66)
TB05	$C_{22}H_{15}BrN_2OS_2$	467.40	205-207	53	56.53(56.53)	3.20(3.23)	5.97(5.99)
TB06	$C_{22}H_{16}N_2O_2S_2$	404.50	199-201	65	65.33(65.32)	3.98(3.99)	6.90(6.93)
TB07	$C_{23}H_{18}N_2OS_2$	402.53	238-240	75	68.62(68.63)	4.50(4.51)	6.96(6.96)
TB08	$C_{22}H_{15}N_3O_3S_2$	433.50	196-198	41	60.95(60.95)	3.47(3.49)	9.67(9.69)
TB09	$C_{22}H_{15}FN_2OS_2$	406.50	229-231	50	65.01(65.00)	3.70(3.72)	6.88(6.89)
TB10	$C_{22}H_{16}N_2OS_2$	388.51	194-196	71	68.00(68.01)	4.13(4.15)	7.20(7.21)

[a] Elemental analyses for C, H and N were within ±0.03% of the theoretical value.
[b] Melting point of the compound at their decomposition
[c] Molecular weight of the compound

Table 3 — Anti-bacterial and anti-fungal activities of the synthesized 4-thiazolidinones-benzothiazole conjugates

Compound code	Microbial species						
	Bacteria					Fungi	
	E.coli	*S.aureus*	*S. typhi*	*P.aeruginosa*	*E. faecalis*	*C. albicans*	*C. tropicalis*
TB01	22-26(15.6)	-	<10(125)	-	12-14(62.5)	16-18(15.6)	<10(125)
TB02	12-15(62.5)	14-18(31.2)	12-14(62.5)	-	15-17(31.2)	10-12(62.5)	14-16(31.2)
TB03	16-19(31.2)	13-15(62.5)	11-14(62.5)	<10(125)	11-13(62.5)	<10(125)	13-15(31.2)
TB04	13-17(62.5)	15-17(31.2)	12-15(62.5)	-	-	12-15(31.2)	<10(125)
TB05	<10(125)	-	<10(125)	11-13(62.5)	-	12-15(31.2)	-
TB06	24-26(15.6)	-	<10(125)	-	<10(125)	17-19(15.6)	12-15(31.2)
TB07	15-18(31.2)	14-18(31.2)	11-14(62.5)	-	-	<10(125)	10-12(62.5)
TB08	<10(125)	-	<10(125)	10-12(62.5)	<10(125)	<10(125)	-
TB09	-	<10(125)	-	-	<10(125)	-	11-13(62.5)
TB10	<10(125)	<10(125)	-	14-17(31.2)	<10(125)	<10(125)	-
Ciprofloxacin	30-32(6.25)	30-33(6.25)	28-31 (6.25)	29-32 (3.12)	27-29 (6.25)	-	-
Fluconazole	-	-	-	-	-	22-25(6.25)	24-27 (6.25)

The value of each compound consisted of 'zone of inhibition range (MIC)' of 03 replicates
Level of significance $P < 0.05$

members of the series with moderate to good MIC values ranging between 15.6–125 µg/mL. The results of antimicrobial screening revealed that among the compounds screened, compounds TB02, TB03, TB04 and TB07 showed moderate activity while compounds TB01 and TB06 displayed good antimicrobial activity. Particularly, compounds such as 3-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-methoxyphenyl) thiazolidin-4-one (TB01) and 3-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-hydroxyphenyl) thiazolidin-4-one (TB06) were found to be the most active candidate against *E. coli* (zone of inhibition up to 22–26 mm at concentration of 15.6 µg/mL) and against *C. albicans* (zone of inhibition up to 16–19 mm at concentration of 15.6 µg/mL) (Fig. 1).

The DNA cleaving ability of compounds TB01, TB06, TB08 and TB09 was studied by agarose gel electrophoresis using plasmid DNA as a substrate[27]. The activity of these compounds was assessed by the conversion of DNA from Form I to Form II or Form III. A concentration-dependent DNA cleavage by TB01 and TB06 along with TB08 and TB09 as negative control were performed. At 10 µM concentration, TB01 and TB06 exhibited efficient nuclease activity whereas no cleavage activity was observed in compounds TB08 and TB09. The present compounds at micromolar concentrations, after 1h incubation with DNA, showed the nucleolytic activities (Fig. 2).

Compounds TB01 and TB06 were carrying electron donating groups (methoxy, hydroxy) on aryl

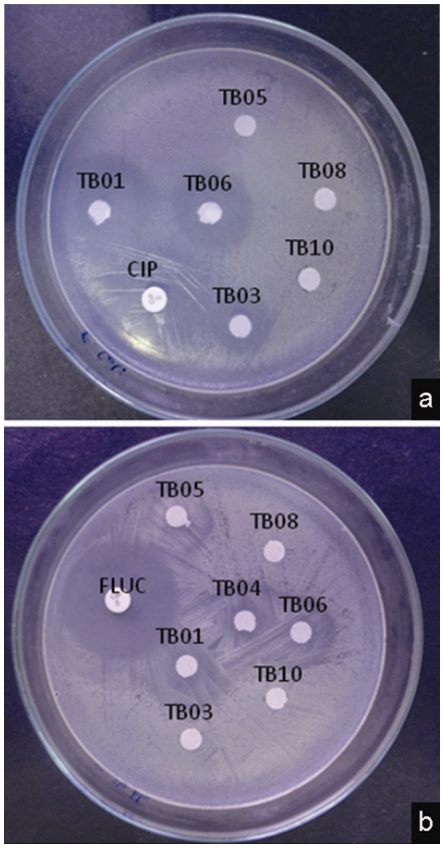


Fig. 1— Antimicrobial activity of 4-thiazolidinones-benzothiazole conjugates (a) Antibacterial activity against E. *coli* with reference to standard drug Ciprofloxacin, TB01, TB03 and TB06 showing good inhibition compare to TB05, TB08 and TB10. (b) Antifungal activity against *C. albicans* with reference to standard drug Fluconazole, TB01, TB04, TB05 and TB06 showing the greater inhibition compare to TB03, TB08 and TB10.

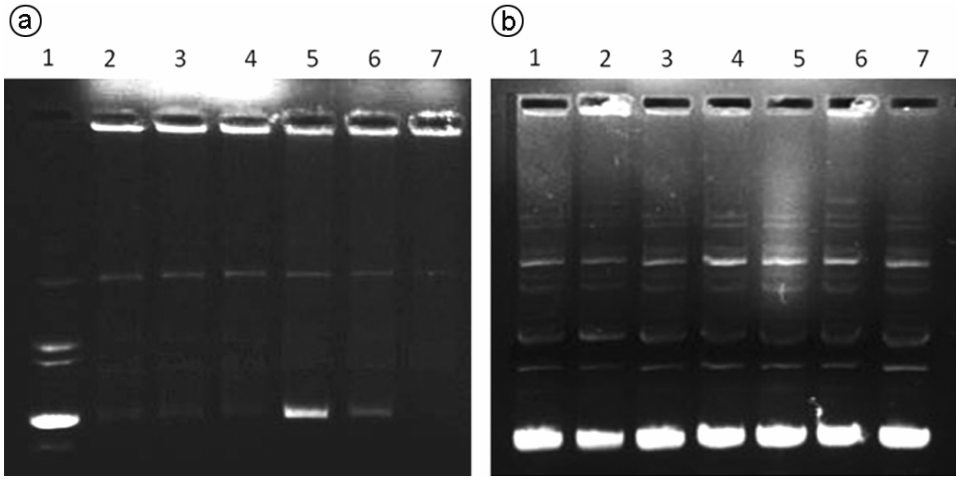


Fig. 2 —Agarose gel electrophoresis patterns of plasmid DNA (200 ng) cleaved by (a) TB01 and TB06 (10-30 µM), after 1h incubation time (concentration dependent) Lane 1: DNA control; Lane 2: 10 µM TB01 + DNA; Lane 3: 20 µM TB01 + DNA; Lane 4: 30 µM TB01 + DNA; Lane 5: 10 µM TB06 + DNA; Lane 6: 20 µM TB06 + DNA; Lane 7: 30 µM TB06 + DNA in buffer (5mM Tris –HCl/50mM NaCl, pH= 7.2 at 25 °C). (b) TB08 and TB09 (10-30 µM), after 1h incubation time (concentration dependent) Lane 1: DNA control; Lane 2: 10 µM TB08 + DNA; Lane 3: 20 µM TB08 + DNA; Lane 4: 30 µM TB08 + DNA; Lane 5: 10 µM TB09 + DNA; Lane 6: 20 µM TB09 + DNA; Lane 7: 30 µM TB09 + DNA in buffer (5mM Tris –HCl/50mM NaCl, pH= 7.2 at 25 °C).

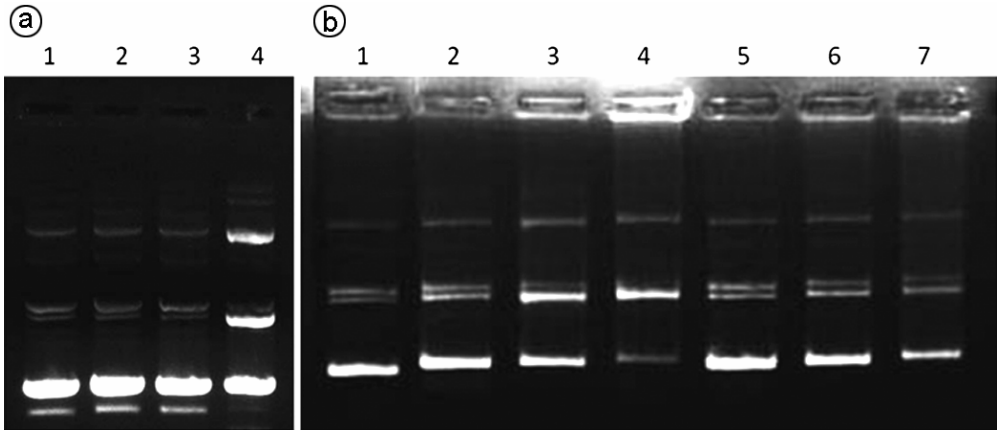


Fig. 3 —Agarose gel electrophoresis pattern for the cleavage of supercoiled DNA (200 ng) by TB01 and TB06 (10 μM), in presence of different radical scavengers. (a) Lane 1: DNA control; Lane 2: NaN_3 + DNA; Lane 3: DMSO + DNA; Lane 4: t-butyl alcohol + DNA; in buffer (5mM Tris –HCl/ 50 mM NaCl, pH= 7.2 at 25°C). (b) Lane 1: DNA control; Lane 2: TB01 + NaN_3 + DNA; Lane 3: TB01+ DMSO + DNA; Lane 4: TB01 + t-butyl alcohol + DNA; Lane 5: TB06 +NaN_3 +DNA; Lane 6: TB06+ DMSO + DNA; Lane 7: TB06 + t-butyl alcohol + DNA in buffer (5mM Tris –HCl/ 50 mM NaCl, pH= 7.2 at 25°C).

ring and showed significant increase in antimicrobial activity against *E. coli* and *C. albicans.* A significant number of drugs and drug candidates in clinical development are halogenated structures. The formation of halogen bonds in ligand-target complexes is recognized as a kind of intermolecular interaction that favourably contributes to the stability of protein-ligand complexes. The insertion of halogen atoms has been used in innumerous cases of hit-to-lead or lead-to-drug conversions[28,29]. Derivatization of TB02 with chloro substitution on phenyl ring exhibited moderate potency (zone of inhibition = 10-18 mm) indicating that substitutions may result in restoration of potency. Whereas, compounds containing other halogen atoms viz., fluoro (TB09), bromo (TB05) substitutions exhibited least potency. Compounds TB08 bearing electron withdrawing group (nitro) and of without derivatization TB10, also exhibited least potency.

Structure–activity relationship (SAR) studies from the results of the antimicrobial activity revealed that conversion of Schiff bases via formation of 4-thiazolidinones-benzothiazole conjugates may contribute for good activity. The greater antimicrobial activity of TB01, TB02, TB03, TB04, TB06 and TB07 may be attributed to the presence of electron donating substituents such as methoxy, hydroxyl and also of chloro substituents on phenyl ring.

Cleavage in plasmid DNA was detected by UV illumination in presence of ethidium bromide (EB), which is the most widely used intercalative agent and fluorescence probe for DNA structure[30]. When the supercoiled (SC) form I of DNA is nicked, it gives rise to an open circular (OC) relaxed form II and further cleavage gives a linear form III. In gel electrophoresis, SC shows the fastest migration, followed by linear form and lastly the OC form.

To investigate the probable mechanism of nuclease activity by TB01 and TB06, comparative reactions were carried out in presence of various radical inhibitors or trappers[31,32] such as singlet oxygen scavenger sodium azide (NaN_3), hydroxyl radical scavengers via; dimethylsulphoxide (DMSO) and t-butyl alcohol (TBA) (Fig. 3 a and b). When the hydroxyl radical inhibitor DMSO and TBA were added to the reaction mixture no evident inhibition of the nuclease activity was observed, suggesting non involvement of hydroxyl radical in the cleavage process. Similarly, the nuclease activity of the compounds TB01 and TB06 did not change in presence of free radical scavenger like NaN_3, thus the presence of singlet oxygen can be ruled out. Therefore, DNA cleavage promoted by TB01 and TB06 might mainly (be or come) through the non-oxidative pathway thus it is very possible that the phosphodiester bond of DNA would have been cleaved by TB01 and TB06 via phosphoryl transfer reactions[33, 34].

These promising findings may exalt the scope of developing these thiazolidinones derivatives of benzothiazole as promising antibacterial and antifungal agents. Further, TB01 and TB06 interacted with plasmid DNA and the electrophoresis pattern obtained showed specific and significant cleavage.

Consequently, they have shown the potential to act as good artificial nuclease mimics. These compounds possess significant potential to act as metal-free nucleolytic agents. Thus, DNA cleavage experiments gave valuable hints with supporting evidence for describing the mechanism of action and hence showed a good correlation between their calculated MIC's and lethality.

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References

1 Yalcin I, Oren I, Sener E, Akin A & Ucarturk N, The synthesis and the structure–activity relationships of some substituted benzoxazoles, oxazolo(4,5-*b*)pyridines, benzothiazoles and benzimidazoles as antimicrobial agents, *Eur J Med Chem*, 27 (1992) 401.

2 Al-Soud Y A, Al-Sadoni H H, Saeed B, Jaber I H, Beni-Khalid M O, Al-Masoudi N A, Tahsin A, Colla P L, Busonera B, Sanna T & Loddo R, Synthesis and *in vitro* antiproliferative activity of new benzothiazole derivatives, *Arkivoc*, 15 (2008) 225.

3 Hout S, Azas N, Darque A, Robin M, Giorgio C D, Gasquet M, Galy J & Timon-David P, Activity of benzothiazoles and chemical derivatives on *Plasmodium falciparum*, *Parasitology*, 129 (2004) 525.

4 Chakole R D, Amnerkar N D, Khedekar P B & Bhusari K P, Synthesis and substituted benzothiazole derivatives of thioquinazoline as anticovulsant agents, *Indian J Heterocyclic Chem*, 15 (2005) 27.

5 Amnerkar N D & Bhusari K P, Synthesis of some thiazolyl aminobenzothiazole derivatives as potential antibacterial, antifungal and anthelmintic agents, *J Enzyme Inhib Med Chem*, 26 (2011) 22.

6 Siddiqui N, Alam M & Siddiqui A A, Synthesis and analgesic activity of some 2-[{4-(Alkyl thioureido) phenyl}sulphonamido]-6-substituted benzothiazoles, *Asian J Chem*, 16 (2004) 1005.

7 Kaur H, Kumar S, Singh I, Saxena K K & Kumar A, Synthesis, Characterization and Biological activity of various substituted Benzothiazole derivatives, *Digest J Nano Bio Str*, 5 (2010) 67.

8 Navarrete-Vazquez G, Paoli P, León-Rivera I, Villalobos-Molina R, Medina-Franco J L, Ortiz-Andrade R, Estrada-Soto S, Camici G, Diaz-Coutiño D, Gallardo-Ortiz I, Martinez-Mayorga M & Moreno-Díaz H, Synthesis, *in vitro* and computational studies of protein tyrosine phosphatase1B inhibition of a small library of 2-arylsulfonylaminobenzothiazoles with anti hyperglycemic activity, *Bioorg & Med Chem*, 17 (2009) 3332.

9 Geronikaki A A, Lagunin A A, Hadjipavlou-Litina D I, Eleftheriou P T, Filimonov D A, Poroikov V V, Alam I & Saxena A K, Computer-aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipoxygenase inhibition, *J Med Chem*, 51 (2008) 1601.

10 Parekh H H, Parikh K A & Parikh A R, Synthesis of some 4-thiazolidinone derivatives as antitubercular agents, *J Sci Islamic Rep Iran,* 15 (2004) 143.

11 Lokhandwala S R & Desai K R, Novel organophosphorus compounds as potential antimicrobial agents, *Phosphorus, Sulfur and Silicon Relat Elements,* 183 (2008) 1264.

12 Terzioglu N, Karali N, Gursoy A, Pannecouque C, Leysen P, Paeshuyse J, Neyts J & Clercq E D, Synthesis and primary antiviral activity evaluation of 3-hydrazono-5-nitro-2-indolinone derivatives, *Arkivoc*, 1 (2006) 109.

13 Balzarini J, Orzeszko-Krzesinska B, Maurin J K & Orzeszko A, Synthesis and anti-HIV studies of 2- and 3-adamantyl-substituted thiazolidin-4-ones, *Eur J Med Chem*, 44 (2009) 303.

14 Singh M, Thakur B, Ray P, Nath G, Gangwar M & Singh S K, Design and Synthesis of Novel Schiff base-benzothiazole hybrids: potential antimicrobials and Epidermal Growth Factor Receptor (EGFR) Inhibitors, *Eur J Med Chem*, (2013), (communicated).

15 Cotton F A, Hazen E E, Jr, Legg M J, Staphylococcal nuclease: proposed mechanism of action based on structure of enzyme-thymidine 3′,5′-bisphosphate–calcium ion complex at 1.5-Å resolution, *Proc Natl Acad Sci USA*., 76 (1979) 2551.

16 Breslow R, Huang D-L, Anslyn E, On the mechanism of action of ribonucleases: dinucleotide cleavage catalyzed by imidazole and Z^{n2+}, *Proc Natl Acad Sci USA*., 86 (1989) 1746.

17 Schmidtchen F P, Berger M, Artificial Organic Host Molecules for Anions, *Chem Rev*, 97 (1997) 1609.

18 Chen X, Wang J, Sun S, Fan J, Wu S, Liu J, Ma S, Zhang L & Peng X, Efficient enhancement of DNA cleavage activity by introducing guanidinium groups into diiron(III) complex, *Bioorg Med Chem Lett*, 18 (2008) 109.

19 Sheng X, Lu X-M, Chen Y-T, Lu G-Y, Zhang J-J, Shao Y, Liu F & Xu Q, Synthesis, DNA-Binding, Cleavage, and Cytotoxic Activity of New 1,7-Dioxa-4,10-diazacyclododecane Artificial Receptors Containing Bisguanidinoethyl or Diaminoethyl Double Side Arms, *Chem Eur J,* 13 (2007) 9703.

20 Shao Y, Sheng X, Li Y, Jia Z-L, Zhang J-J, Liu F & Lu G-Y, DNA Binding and Cleaving Activity of the New Cleft Molecule N,N′-Bis(guanidinoethyl)-2,6-pyridinedicarboxamide in the Absence or in the Presence of Copper(II), *Bioconjugate Chem*, 19 (2008) 1840.

21 Sheng X, Lu X-M, Zhang J-J, Chen Y-T, Lu G-Y, Shao Y, Liu F & Xu Q, Synthesis and DNA Cleavage Activity of Artificial Receptor 1,4,7-Triazacyclononane Containing Guanidinoethyl and Hydroxyethyl Side Arms, *J Org Chem*, 72 (2007) 1799.

22 Shao Y, Ding Y, Jia Z-Li, Lu X-M, Ke Z-H, Xu W-H & L G-Y, Synthesis and DNA cleavage activity of 2-hydrazinyl-1,4,5,6-tetrahydropyrimidine containing hydroxy group, *Bioorg Med Chem*, 17 (2009) 4274.

23 Audrieth L F, Scott E S & Kippur P S, Hydrazine derivatives of the carbonic and thiocarbonic acids. I. The preparation and properties of thiocarbohydrazide, *J Org Chem*, 19 (1954) 733.

24 Wayne P A, National Committee for Clinical Laboratory Standards, NCCLS Approved standard M27-A. USA, 1997.

25 Tuite J, *Plant Pathological Methods, Fungi and Bacteria,* (Burgess Publishing Company, Minneapolis) 1969, 101.

26 Yang Q, Xu J, Sun Y, Li Z, Li Y & Qian X, Hydrolysis of plasmid DNA and RNA by amino alkyl naphthalimide as metal-free artificial nuclease, *Bioorg Med Chem Lett*, 16 (2006) 803.

27 Fernandes L, Fischer F L, Ribeiro C W, Silveira G P, Sá M M, Nome F & Terenzi H, Metal-free artificial nucleases based on simple oxime and hydroxylamine scaffolds, *Bioorg Med Chem Lett*, 18 (2008) 4499.

28 Bonnefous C, Payne J E, Roppe J, Zhuang H, Chen X, Symons K T, Nguyen P M, Sablad M, Rozenkrants N, Zhang Y, Wang L, Severance D, Walsh J P, Yazdani N, Shiau A K, Noble S A, Rix P, Rao T S, Hassig C A & Smith N D, Discovery of inducible nitric oxide synthase (iNOS) inhibitor development candidate KD7332, part 1: Identification of a novel, potent, and selective series of quinolinone iNOS dimerization inhibitors that are orally active in rodent pain models. *J Med Chem*, 52 (2009) 3047.

29 Leite A C, Moreira D R, Cardoso M V, Hernandes M Z, Alves Pereira V R, Silva R O, Kiperstok A C, Lima Mda S & Soares M B, Synthesis, Cruzain docking, and *in vitro* studies of aryl-4-oxothiazolylhydrazones against *Trypanosoma cruzi*, *Chem Med Chem,* 2 (2009) 1339.

30 Wilson W D, Ratmeyer L, Zhao M, Strekowski L & Boykin D, The Search for Structure-Specific Nucleic Acid-Interactive Drugs: Effects of Compound Structure on RNA versus DNA Interaction Strength, *Biochemistry*, 32 (1993) 4098.

31 Thyagarajan S, Murthy N N, Narducci A, Sarjeant A, Karlin K D & Rokita S E, Selective DNA Strand Scission with Binuclear Copper Complexes: Implications for an Active Cu_2-O_2 Species, *J Am Chem Soc*, 128 (2006) 7003.

32 Perrin D M, Mazumder A, Sigmanin D S, Cohn W & Moldave K, *Progress in nucleic acid chemistry and molecular biology* (Academic Press, New York and Orlando) 52, 1996, 123.

33 Muche M-S & Göbel M W, Bis(guanidinium) Alcohols as models of staphylococcal nuclease: substrate binding through ion pair complexes and fast phosphoryl transfer reactions, *Angew Chem Int Ed*, 35 (1996) 2126.

34 Sheng X, Lu X-M, Zhang J-J, Chen Y-T, Lu G-Y, Shao Y, Liu F, Xu Q, Synthesis and DNA cleavage activity of artificial receptor 1,4,7-triazacyclononane containing guanidinoethyl and hydroxyethyl side arms, *Org Chem* 72 (2007) 1799.