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ORIGINAL PAPER

Influence of substituent effects on spectroscopic properties and antimicrobial activity of 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives

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Abstract 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives (HL_n) have been synthesized and characterized by elemental analysis, spectra (IR, electronic and ^1H NMR). The IR spectral data indicate that the compounds can exist in two resonance structures. The synthesized ligands were screened for their antimicrobial activity against four bacterial species, two Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and two Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) as well as against some species various of fungi; *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium italicum* and *Fusarium oxysporium.* The results showed that most these ligands are good antibacterial agents against *B. cereus* and *S. aureus* and antifungal agents against *A. niger* and *F. oxysporium*. HL_3 was found to be the most effect compound against all tested microorganisms. The size of clear zone were ordered as follows *p*-($OCH_3 < CH_3 < H < Cl < NO_2$) as expected from Hammett's constant σ^R.

Keywords Azo rhodamine derivatives · Antibacterial and antifungal activities

Introduction

Azo compounds based on rhodanine were synthesized as potential medicinal preparations (El-Bindary et al. 2001) and can also be used as analytical reagents (Stephen and Townshend 1965; Alfonso and Ariza 1981). It is known that rhodanine plays an important role in biological reactions (Wang et al. 2001) e.g. in the inhibition of *Mycobacterium tuberculosis* (Shehatta and El-Bindary 1994). Chemical properties of rhodanines and its derivatives are of interest due to coordination capacity and their use as metal extracting agents (El-Sonbati and El-Bindary 2000) and also widely prepared and studied several metal complexes of rhodanine azodyes due to their unusual magnetic properties and relevance to biological system (El-Bindary and El-Sonbati 2000; El-Sonbati et al. 2002). Rhodanine and its derivatives are known to possess biological activities such as antimiotic, antidiabetic, hypocholesterolemic, antiperlipemic, antiviral and antidiabetic in nature and rhodanine derivatives as hepatitis C virus (HCV) protease inhibitor are also reported (Momose et al. 1991; Sudo et al. 1997; Enchev et al. 2002; Moorthy et al. 2010).

Ligands with potential sulfur, oxygen and nitrogen donors, such as rhodanine and its derivatives are quite interesting which have gained special attention in the last decade, not only because of the structural chemistry and their importance in medical chemistry, but also because these materials are used as a drugs and they are reported to possess a wide variety of antimicrobial activities against bacteria and fungi. They are also become a useful model for bioinorganic processes, which has many biochemical and pharmacological (Gao et al. 2000; Sortino et al. 2007; El-Sonbati et al. 2012a). To the best of our knowledge, no efforts were done to study the microbial activity for 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives (HL_n).

The aim of the present work is to synthesize 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives (HL_n) and test the antimicrobial activity against two Gram positive bacteria (*Bacillus cereus* and *Staphylococcus*

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aureus) and two Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) in addition to different types of fungi; *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium italicum* and *Fusarium oxysporium.*

Experimental

Preparation of the ligand 2-thioxo-4-thiazolidinone according to El-Sonbati and El-Dissouky (1987); El-Bindary et al. (2002); El-Sonbati et al. (2004). The standard chemical aniline and 4-alkyl anilines (alkyl: CH_3, OCH_3, Cl, NO_2; Aldrich chemical Co.) were used without any further purification. The experimental technique has been described previously (El-Bindary and El-Sonbati 1997; El-Bindary and El-Sonbati 1999; El-Sonbati and El-Bindary 2000; Shoair et al. 2000; Nikalje et al. 2011).

The novel 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives (HL_n) were prepared from aniline or p-substituted anilines (10 mmol) was dissolved in hydrochloric acid (20 mmol/25 ml distilled H_2O). The hydrochloric compound was diazotized below −5 °C with a solution of sodium nitrite (0.8 g, 10 mmol, 30 ml distilled H_2O). The diazonium chloride was coupled with an alkaline solution of 2-thioxo-4-thiazolidinone (1.33 g, 10 mmol) in 20 ml of ethanol. The crude dye was collected by filtration and was crystallized from ethanol/DMF; then dried in vacuum desiccators over P_2O_5.

The resulting formed ligands are: 5-(4′-methoxyphenylazo)-2-thioxothiazolidin-4-one (HL_1), 5-(4′-methylphenylazo)-2-thioxothiazolidin-4-one (HL_2), 5-(4′-phenylazo)-2-thioxothiazolidin-4-one (HL_3), 5-(4′-chlorophenylazo)-2-thioxothiazolidin-4-one (HL_4) and 5-(4′-nitrophenylazo)-2-thioxothiazolidin-4-one (HL_5).

Microbiological investigation

For this investigation the agar well diffusion method was applied (Elzahany et al. 2008; Alghool et al. 2010). The antibacterial activities of the investigated compounds were tested against two local Gram positive bacterial isolates (*B. cereus* and *S. aureus* local isolates) and two local Gram negative bacterial isolates (*E. coli* and *K. pneumoniae*) on nutrient agar medium. Also, it was tested against some kinds of local fungal isolates fungi (*A. niger*, *P. chrysogenum*, *P. italicum* and *F. oxysporium*) on Dox agar medium. The concentrations of each solution were 150, 100, 50 and 30 μg/ml. By using a sterile cork borer (10 mm diameter), wells were made in agar medium plates previously seeded with the test organism. 200 μl of each compound was applied in each well. The agar plates were kept at 4 °C for at least 30 min. to allow the diffusion of the compound to agar medium. The plates were then incubated at 37 or 30 °C for bacteria and fungi, respectively. Penicillin and miconazole were used as antibacterial and antifungal respectively. The diameters of inhibition zone were determined after 24 h for bacteria and 7 days for fungi.

Measurements

Elemental microanalyses of the separated ligands for C, H, and N were performed in the Microanalytical Center, Cairo University, Egypt. The analyses were repeated twice to check the accuracy of the analyzed data. The ^{1}H-NMR spectrum was obtained with a JEOL FX90 Fourier transform spectrometer with DMSO-d_6 as the solvent and TMS as an internal reference. Infrared spectra were recorded as KBr pellets using a Pye Unicam SP 2000 spectrophotometer. Ultraviolet–Visible (UV–Vis) spectra of the compounds were recorded in nuzol solution using a Unicom SP 8800 spectrophotometer.

Results and discussion

The chemical structure of the ligands was elucidated by elemental analyses (Table 1), IR electronic and ^{1}H NMR spectra. The optical absorption measurements showed many absorption bands according to transition from bonding to antibonding molecular orbital.

The ^{1}H NMR spectroscopy was used to differentiate stereoisomers. El-Sonbati and coworkers investigated the NMR spectra of azo rhodanine and its derivatives with various transition metal salts (Mubarak et al. 2007; Diab et al. 2010; El-Sonbati et al. 2011a; El-Sonbati et al. 2012b). The ^{1}H NMR spectra are in agreement. Signal for CH (∼4.42 ppm), favoring formation of an intramolecular hydrogen bond with the N=N (azodye) group. Electron-withdrawing substituents reduce the intramolecular hydrogen bond as indicated by the marked shift of the hydroxyl signal to higher field in the *p*-NO_2 and *p*-Cl compounds. Electron-donating substituents give the opposite effect, arising from the increasing basicity of the azo-nitrogen. The broad signals assigned to the OH protons at ∼11.36–11.88 ppm are not affected by dilution. The previous two protons disappear in the presence of D_2O. Absence of –CH proton signal of the ligand moiety indicated the existence of the ligand in the azo-enol form. According to Mubarak et al. (2007); Diab et al. (2010); El-Sonbati et al. (2011a); El-Sonbati et al. (2012b), hydrogen bonding leads to a large deshielding of the protons. The shifts are in the sequence: *p*-(NO_2 > Cl > H > OCH_3 > CH_3). In the meantime, the ^{1}H NMR of the HL_1/HL_2 exhibits signals at δ (ppm) [3.3 (s, 3H, CH_3)]/[3.9 (s, 3H, OCH_3)]. The aromatic protons have resonance at 7.10–7.45 ppm for the ligands.

Table 1 Analytical data of azorhodanine derivatives

Compound	Empirical formula	Yield %	M.p.°C	Calc. (Exp.) %		
				C	H	N
HL_1	$C_{10}H_9N_3O_2S_2$	37.45	221	44.93	3.39	15.72
	Red			(44.82)	(3.25)	(15.85)
HL_2	$C_{10}H_9N_3OS_2$	47.81	231	47.79	3.61	16.72
	Dark orange			(47.88)	(3.76)	(16.61)
HL_3	$C_9H_7N_3OS_2$	42.19	237	45.55	2.97	17.71
	Pale yellow			(45.68)	(2.80)	(17.85)
HL_4	$C_9H_6N_3OS_2Cl$	51.37	248	39.78	2.23	15.46
	Light orange			(39.65)	(2.35)	(15.58)
HL_5	$C_9H_6N_4O_3S_2$	66.087	245	38.29	2.14	19.85
	Dark yellow			(38.42)	(2.25)	(19.98)

On the basis of all the above spectral data, an internally hydrogen boned azo-enol structure has been proposed for the ligand (Fig. 1).

The electronic absorption spectra of the ligands exhibit mainly five bands (A–D, F). The band A located at 26,360–26,280 cm^{-1} can be assigned to the n−π* transition of the CS group. The band B within 30,560–30,260 cm^{-1}; can be assigned to n−π* transition within the CO group. The band C within 32,980–33,180 cm^{-1} could be assigned to the H-bonding and association. The band D located at 40,

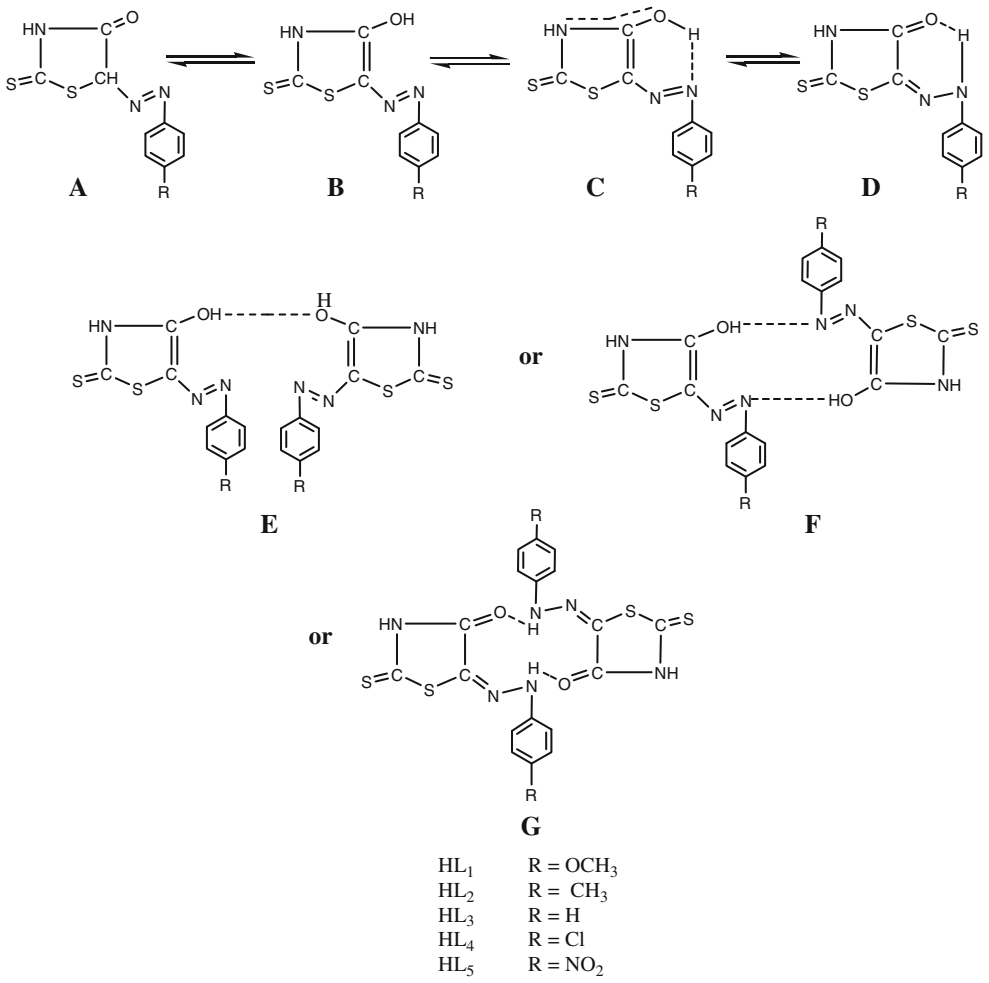

Fig. 1 Structure of ligands (HL_n)

Table 2 Anti-bacterial activity data of HL_n

Compound	Concentration	*Gram positive bacteria		*Gram negative bacteria	
		Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*
HL_1	50 μg/ml	3	6	1	−ve
	100 μg/ml	4	7	−ve	−ve
	150 μg/ml	5	6	2	2
HL_2	50 μg/ml	3	4	−ve	−ve
	100 μg/ml	5	4	−ve	−ve
	150 μg/ml	5	5	2	−ve
HL_3	50 μg/ml	9	4	−ve	−ve
	100 μg/ml	15	7	1	−ve
	150 μg/ml	12	7	1	1
HL_4	50 μg/ml	4	4	1	−ve
	100 μg/ml	7	4	−ve	−ve
	150 μg/ml	4	5	1	1
HL_5	30 μg/ml	6	4	−ve	−ve
	50 μg/ml	6	8	1	1
Penicillin	50 μg/ml	1	2	1	−ve
	100 μg/ml	3	2	3	−ve
	150 μg/ml	3	2	3	−ve

* The results were recorded as the diameter of inhibition zone (mm)

250–3,990 cm^{-1} could be assigned to Ph–Ph*, $\pi-\pi^*$ corresponding to the aromatic system. The band F located at 29,620–29,350 cm^{-1}. These latter bands can be assigned to phenyl rings overlapped by composite broad $\pi-\pi^*$ of azo structure. The band B transition disappears with the simultaneous appearance of new bands, being attributed to $\pi-\pi^*$ (C=C) as sequences of enolization. Furthermore, the band A transition shifts slightly to lower energy and remains almost constant.

In general, most of the azo compounds give spectral localized bands in the region 47,620–34,480 cm^{-1} and 31,250–2,730 cm^{-1}. The first region is due to the absorption of the aromatic ring compared to ^1B_b and ^1L_b mono substituted benzene and the second region is due to the conjugation between the azo group and the aromatic nuclei with internal charge transfer resulting from π-electron migration to the diazo group from the electron donating substituents. The *p*-substituent increases the conjugation with a shift to a longer wavelength (El-Dissouky et al. 2001). The substituent effect is related to the Hammett's constant values (Mohmond et al. 1985; Mubarak et al. 2007; El-Sonbati et al. 2010a). For azo benzene and aryl azo benzene derivatives, as the possibilities of the mesmerism became greater, the stabilization of the excited state is increased relative to that of the ground state and a bathochromic shift of the absorption bands follows. One way of explaining this result is by means of the M. O theory (Jean 2004), which shows that the energy terms of the molecular orbital became more closely spaced as the size of the conjugated system increases. Therefore with every additional conjugated double bond the energy difference between the highest occupied and the lowest vacant π-electron level became smaller and the wavelength of the first absorption band which corresponds to this transition is increased. The azo group can act as a proton acceptor in hydrogen bonds. The role of hydrogen bonding in azo aggregation has been accepted for some time.

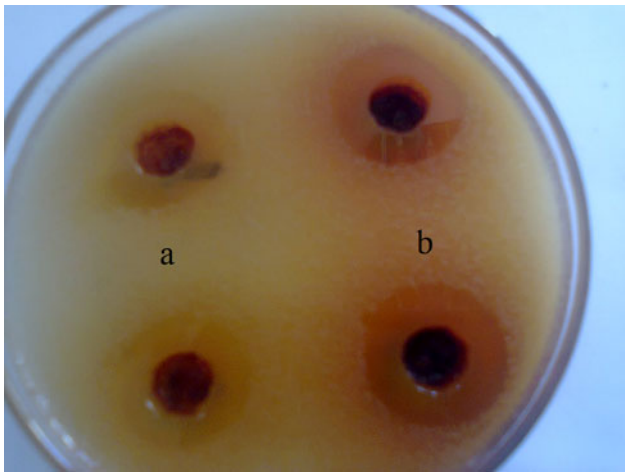

Fig. 2 Effect of HL_1 on growth of *Bacillus cereus* on nutrient agar medium using concentration $a = 50$ μg/ml, $b = 150$ μg/ml

The infrared spectra of HL_n give interesting results and conclusions. The ligands gives two bands at ~3,200–3,040 cm^{-1} due to asymmetric and symmetric stretching vibrations of N–H group and intramolecular hydrogen bonding NH···O systems (Fig. 1d), respectively. When the OH group (Fig. 1c) is involved in intramolecular hydrogen bond, the O···N and N···O bond distances are the same. But, if such mechanism is happened in case of intermolecular hydrogen bond, the O···O and O···N bond distances are differ.

The broad absorption of a band located at ~3,400 cm^{-1} is assigned to *v*OH. The low frequency bands indicate that the hydroxy hydrogen atom is involved in keto ⇔ enol (A⇔B) tautomerism through hydrogen bonding (Fig. 1c). Bellamy (1958) made detailed studies on some carbonyl compounds containing –NH-group. The Δ*v*NH values were used to study the phenomena of association.

On the other hand, the OH group (Fig. 1b) exhibits more than one absorption band. The two bands located at 1,330 and 1,370 cm^{-1} are assigned to in-plane deformation and that at 1,130 cm^{-1} is due *v*C–OH.

However, the 860 cm^{-1} band is probably due to the out-of-plane deformation of the –OH group. On the other hand, the two bands located at 650 and 670 cm^{-1} are identified as δC=O and NH.

Similar to the other investigated compounds, the different modes of vibrations of C–H and C–C band are identified by the presence of characteristic bands in the low frequency side of the spectrum in 600–200 cm^{-1}.

The infrared spectra of ligands shows medium broad band located at ~3,460 cm^{-1} due the stretching vibration of some sort of hydrogen of hydrogen bonding. El-Sonbati et al. (2010b); Mubarak et al. (2007); El-Sonbati et al. (2011a); El-Sonbati et al. (2011b) made detailed studies for the different types of hydrogen bonding which are favorable to exist in the molecule under investigation:

1. Intramolecular hydrogen bond between the nitrogen atom of the –N=N– system and hydrogen atom of the hydroxy hydrogen atom (Fig. 1c). This is evident by the presence of a broad band centered at 3,460 cm^{-1}.
2. Hydrogen bonding of the OH···N type between the hydroxy hydrogen atom and the N-ph group (Fig. 1c).
3. Intermolecular hydrogen bonding is possible forming cyclic dimer through NH···O=C (G), OH···N=N (F) or OH···OH (E) (Fig. 1).

The presence of broad band located at ~3,200 cm^{-1} is strong indication by *v*NH (Fig. 1d). In general, the low frequency of such region from its normal position is, again due to hydrogen bond property gathered with keto ⇔ enol tautomerism.

In general, hydrogen bonding involving both NH and OH groups are proton donors and both –N and –O atoms are proton acceptors. It is of interest since much multiplicity of proton donor and acceptor sites are prevalent in biological systems. Both intra- and intermolecular OH···N and NH···O may form leading to a number of structures in simultaneous equilibrium.

Table 3 Anti-fungal activity data of HL_n

Compound	Concentration	**Aspergillus niger*	**Fusarium oxysporum*	**Penicillium chrysogenum*	**Penicillium italicum*
HL_1	50 μg/ml	1	4	−ve	1
	100 μg/ml	3	1	−ve	2
	150 μg/ml	2	4	−ve	−ve
HL_2	50 μg/ml	−ve	2	−ve	−ve
	100 μg/ml	2	−ve	−ve	−ve
	150 μg/ml	2	2	−ve	−ve
HL_3	50 μg/ml	7	4	2	4
	100 μg/ml	7	4	14	5
	150 μg/ml	15	7	6	4
HL_4	50 μg/ml	2	−ve	2	2
	100 μg/ml	2	−ve	4	4
	150 μg/ml	4	−ve	4	3
HL_5	30 μg/ml	5	−ve	7	8
	50 μg/ml	3	2.5	6	4
Miconazole	50 μg/ml	1	2	2	1
	100 μg/ml	3	3	2	1
	150 μg/ml	4	3	2	2

* The results were recorded as the diameter of inhibition zone (mm)

Again the three bands located at 1,380, 1,340 and 1,310 cm^{-1} identified as δOH gathered with the two bands at 1,240 cm^{-1} assigned as νC-O are strong indication to keto ⇔ enol equilibria. The presence of a medium band at ~1,605 cm^{-1} assigned to νC=N illustrates the tracing of keto structure (Fig. 1d).

Microbiological investigation

The antimicrobial activity of HL_n ligand was tested against bacteria and fungi; we used more than one test organism to increase the chance of detecting their antimicrobial activities. The used organisms in the present investigations included two Gram positive bacteria (*B. cereus* and *S. aureus*) and two Gram negative bacteria (*E. coli* and *K. pneumoniae*) in addition to different kinds of fungi (*A. niger*, *P. chrysogenum*, *P. italicum* and *F. oxysporium*).

The results of the antibacterial activities of the synthesized compounds are recorded in Table 2. All the used HL_n were found to have antibacterial activity against Gram positive bacteria namely; *B. cereus* (inhibition zone of HL_3 = 15 mm at concentration = 100 μg/ml) and (inhibition zone of HL_1 = 3 and 5 mm at concentration = 50 and 150 μg/ml respectively in Fig. 2) and *S. aureus* (inhibition zone of HL_3 = 4 and 7 mm at concentration = 50 and 150 μg/ml respectively), whereas the HL_n is more active against *B. cereus* and *S. aureus* when comparing with penicillin. But low effects were recorded against Gram negative bacteria. Other investigators (Chen et al. 2010) recorded that a class of chalcone and rhodanine-3-acetic acid moieties presented high potency against Gram-positive bacterium (*S. aureus*) and many of these compounds did not inhibit the growth of Gram-negative bacteria (*E. coli*).

The results of the antifungal activities of the synthesized compounds are recorded in Table 3. The results of the examination of antifungal activity of HL_n ligands (Table 3) revealed that the ligands are moderately toxic against fungi (Donald 1997; Metwally et al. 2010), while HL_3 and HL_5 are more toxic against fungi. HL_3 is more active than miconazole against *A. niger*, *P. chrysogenum*, *P. italicum* and *F. oxysporium.* Our results are similar to Habib et al. (1997) who studied the antimicrobial activities of some rhodanine derivatives and they revealed that the most pronounced activity was the antifungal activity against *A. niger* and *Penicillium* sp.

The HL_3 was found to have high antibacterial activity against *B. cereus*, *S. aureus* and antifungal effect against *A. niger*, *P. chrysogenum*, *P. italicum* and *F. oxysporium*, whereas the HL_5 is more active than HL_1, HL_2 and HL_4 against *B. cereus*, *S. aureus*, *A. niger* (inhibition zone of HL_4 = 2 mm at concentration = 50 μg/ml and inhibition zone of HL_5 = 5 mm at concentration = 30 μg/ml in Fig. 3) and *P. chrysogenum.*

As shown in Tables 2 and 3, the values of inhibition zone for ligands (HL_n) is related to the nature of the *p*-substituent as they increase according to the following order *p*-(NO_2 > Cl > H > CH_3 > OCH_3) (Diab et al. 2010; El-Sonbati et al. 2010b; El-Sonbati et al. 2011a; El-Ghamaz et al. 2011). This can be attributed to the fact that the effective charge experienced by the d-electrons increased due to the electron withdrawing *p*-substituent (HL_4 and HL_5) while it decreased by the electrons donating character of (HL_1 and HL_2). This is in accordance with that expected from Hammett's constant σ^R as shown in Figs. 4,

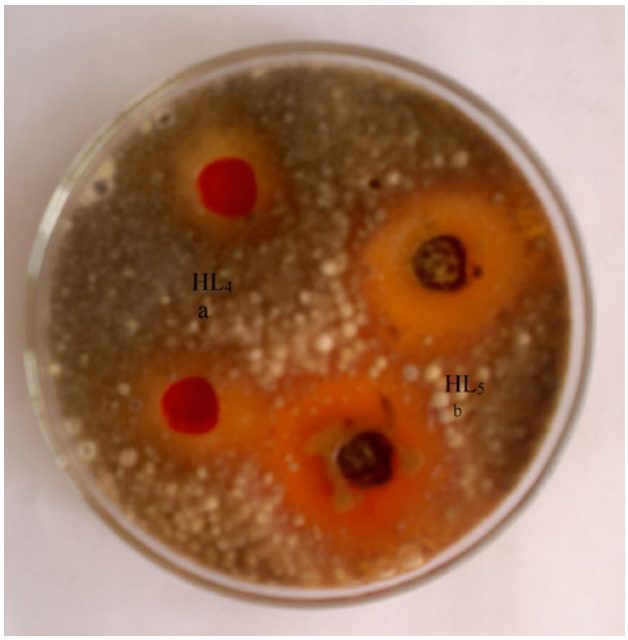

Fig. 3 Effect of HL_4 and HL_5 on growth of *Aspergillus niger* on Dox agar medium using concentration *a* = 100 μg/ml, *b* = 30 μg/ml

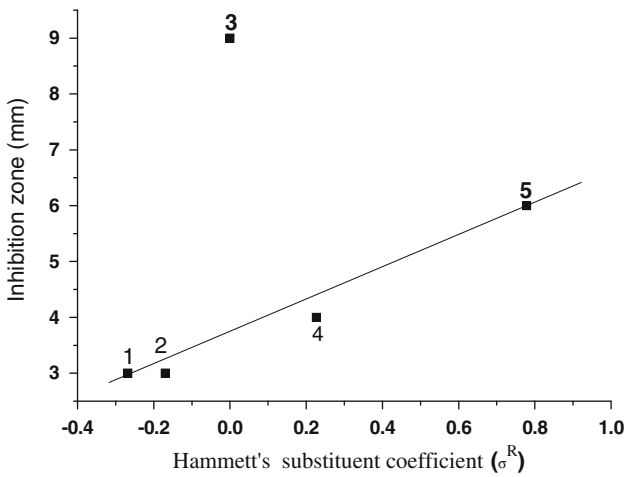

Fig. 4 The relation between Hammett's substitution coefficient (σ^R) versus inhibition zone (mm) (In case of using concentration = 50 μg/ml against *Bacillus cereus*)

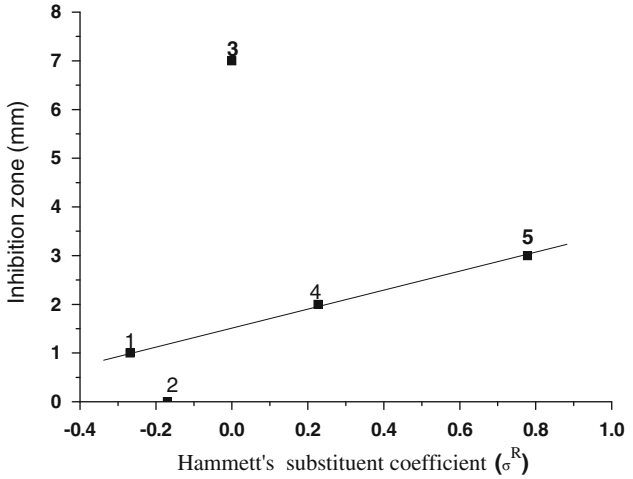

Fig. 5 The relation between Hammett's substitution coefficient (σ^R) versus inhibition zone (mm) (In case of using concentration = 50 μg/ml against *Aspergillus niger*)

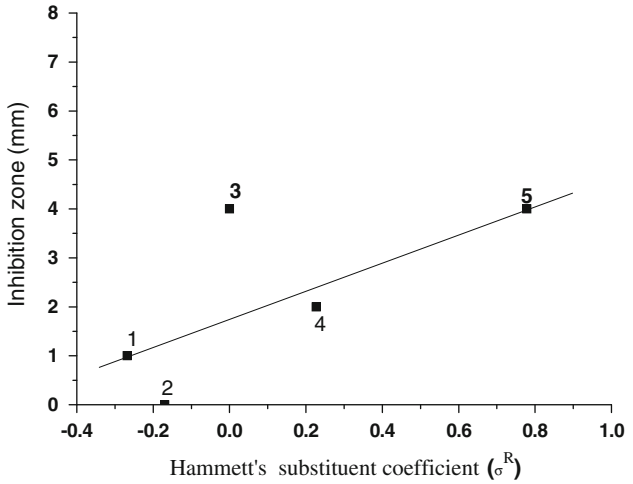

Fig. 6 The relation between Hammett's substitution coefficient (σ^R) versus inhibition zone (mm) (In case of using concentration = 50 μg/ml against *Penicillium italicum*)

5 and 6 correlates the values of inhibition zone (mm) with σ^R, it is clear that these values increase with increasing σ^R. It is important to note that the existence of a methyl and/or methoxy group enhances the electron density on the coordination sites and simultaneously decreases the values of inhibition zone.

Conclusions

The present paper reports on the synthesis of 5-(4′-substituted phenylazo)-2-thioxothiazolidinone derivatives (HL_n) and characterized by elemental analysis, IR, and ^{1}H NMR spectra. The antimicrobial activity was tested against *B. cereus*, *S. aureus*, *E. coli*, *K. pneumoniae, A. niger, F. oxysporum, P. chrysogenum* and *P. italicum.* The antimicrobial tests proved that both tested compounds have antibacterial activity on *B. cereus* and *S. aureus* and antifungal activity on *A. niger* and *P. italicum*.

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