Res Chem Intermed
DOI 10.1007/s11164-012-1001-3

Rhodanine-based biologically active molecules: synthesis, characterization, and biological evaluation

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Received: 27 September 2012 / Accepted: 23 December 2012

Abstract To investigate the antimicrobial properties of the rhodanine (2-thioxo-4-thiazolidinone) structure, several 2-[(5*Z*)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-*N*-phenylacetamide derivatives were synthesized by use of an efficient procedure. Variation of the functional group on the 5-benzylidine ring of rhodanine led to compounds containing a 2-thioxo-4-thiazolidinone group attached to *N*-phenyl acetamide. The chemical structures of the compounds were confirmed by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy, ESI mass spectrometry, and elemental analysis. The antibacterial and antifungal activity of the compounds were tested, at seven concentrations, against Gram-positive bacterial strains (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922), Gram-negative bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 11774), and fungal strains (*Candida albicans* ATCC 66027 and *Aspergillus niger* ATCC 6275), by use of the Kirby Bauer disk-diffusion technique and the serial broth dilution technique. The results obtained were compared with those for reference drugs. Relationships between structure and their antimicrobial activity are discussed.

Keywords 2-Thioxo-4-thiazolidinone · SAR study · Antibacterial activity · Antifungal activity

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Published online: 22 January 2013

Introduction

Treatment of microbial infections has become a challenging task because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens [1]. Despite the large number of antibiotics available for medical use, design and synthesis of new potent antimicrobial agents is still required because of acquired resistance against old and new antibiotics [2]. This has led to a potential approach to overcome the resistance problem and to design innovative agents with a diverse structures and different mechanisms of action from those of existing drugs, so that no cross resistance will occur with current therapeutics [3].

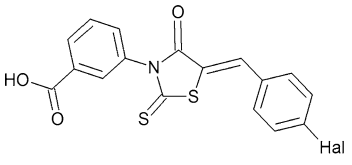
Thiazolidines are important structural fragments in modern medicinal chemistry because of their wide range of pharmacological activity and their affinity for a variety of biological targets. Among thiazolidines, a variety of 4-thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B, a precursor acting during the biosynthesis of peptidoglycan [4], an essential component of the cell wall of both Gram-positive and Gram-negative bacteria. Some thiazolidine derivatives, especially 4-thiazolidinones, are PPAR-receptor agonists with hypoglycaemic, antineoplastic, and anti-inflammatory properties [5], for example complex COX-2/5-LOX inhibitors [6, 7] with anti-inflammatory action, and UDP-MurNAc/L-Ala ligase inhibitors with antimicrobial activity [8]. Thiazolidinones also have high affinity for such biological targets as JNK-stimulating phosphatase-1 (JSP-1) [9] and tumour necrosis factor TNF-α [10]. Recently, 2-thioxo-4-thiazolidinone (rhodanine)-based compounds have been reported to have a wide range of biological activity, for example antibacterial [8, 11], antifungal [12], antidiabetic [13], antitubercular [14, 15], and anti-HIV [16].

As part of our ongoing research on the development of new active antimicrobials [17], and focussing on the above observations, we decided to synthesize a variety of 2-thioxo-4-thiazolidinone-based *N*-phenyl acetamides. In this work, the structural variation was produced by introducing different arylidene substituents at position 5 of the 2-thioxo-4-thiazolidinone structure. The purpose of this investigation was to determine the effect of different arylidene substituents on biological activity, because these substituents have been used as bioactive groups on heterocyclic structures [18–20] (Fig. 1).

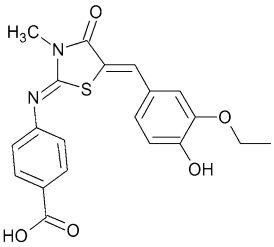
Results and discussion

Chemistry

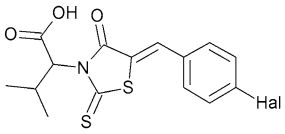
The 2-[(5*Z*)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-*N*-phenyl acetamide derivatives discussed were synthesized in several stages by use of known synthetic procedures [21] (Schemes 1 and 2) which included such reactions as condensation and alkylation. The starting material, 2-thioxo-4-thiazolidinone, was converted into the potassium salt by reaction with potassium hydroxide in ethanol [22]. The potassium salt was then alkylated with 2-chloro-*N*-phenylacetamide to form 2-(4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-*N*-phenylacetamide (int. **5**). This



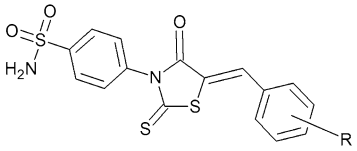
JSP- 1 inhibitors



Integrin $\alpha_v\beta_3$ antagonist



Inhibitors of apoptic proteins interaction Bcl-Xl and BH_3



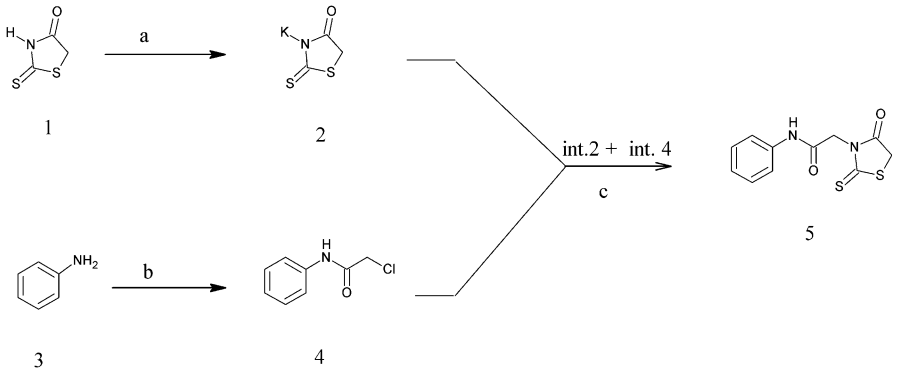
Antimicrobial agents

Fig. 1 Structures of bioactive rhodanines with substitution at active C_3 and N_5 positions

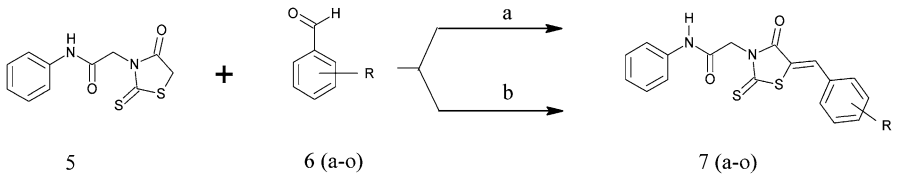
reaction was performed in a mixture of ethanol and DMF as solvent, with potassium iodide and carbonate. The method is relatively simple and convenient, and does not require any additional reagent, as in 4-azolidinone-3-acetic acid carboxylic group functionalization [22]. The resulting intermediate, on Knoevenagel condensation with different benzaldehyde derivatives in the presence of piperidine in ethanol, gives the final compounds, **7a–o**; **7a–o** can also be synthesized in glacial acetic acid by use of sodium acetate. Reaction of benzaldehydes at the 5 position of rhodanine in both media revealed that yield and reaction time were better when the reaction was performed under the basic conditions. Thus, for synthesis of the other compounds basic conditions only were preferred.

The structures of **7a–o** were determined by use of spectral data. ^{1}H NMR spectra of the final compounds contained singlets in the downfield region in the range (δ) 8.53–9.10 ppm, attributed to the –NH lactam proton of the amide. The $-CH_2$ alkyl fragment at the N_3 position of the final compounds contained a sharp singlet in the range 3.25–3.54 ppm. The chemical shift of the methylidene group of the 5-benzylidine derivatives was observed at weak magnetic field, i.e., in the range 7.90–7.98 ppm, and clearly indicated formation of the *Z* isomer in the Knoevenagel reaction [23] (Fig. 2).

The ^{13}C NMR spectra of **7a–o** contained signals of two carbonyl carbon atoms in the downfield region, i.e., 165.98–168.93 ppm. Moreover, exocyclic carbon (=CH) was also observed in the downfield region, i.e., 135.11–139.34 ppm, confirming completion of the Knoevenagel condensation. The other aromatic carbons were



Scheme 1 Synthesis of 2-(4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-*N*-phernlacetamide; *a* KOH, EtOH, R.T., 1h. *b* Chloro acetyl chloride, acetone/benzene, K_2CO_3, 5 h reflux. *c* KI, K_2CO_3, DMF + EtOH (l:l), reflux 4h



Scheme 2 Synthesis of final compounds 7 (**a**–**o**): *a* EtOH, piperidine, reflux, 5–7 h. *b* gla CH_3COOH, anhy. CH,COONa (2 eq.). reflux, 8–12 h: 7 (**a**–**o**): R = **7a** H. **7b** 4–Cl, **7c** 3–Cl, **7d** 2–Cl, **7c** 2,4–Cl, **7t** 3–F, **7g** 4–Br, **7h** 4–Me, **7i** 3–Mc, **7j** 4–OCH_3, **7k**3-OCH_3, **71** 3,4–OCH_3, **7m** 4–NO_2, **7n** 3–NO_2, **7o** 4–CF_3

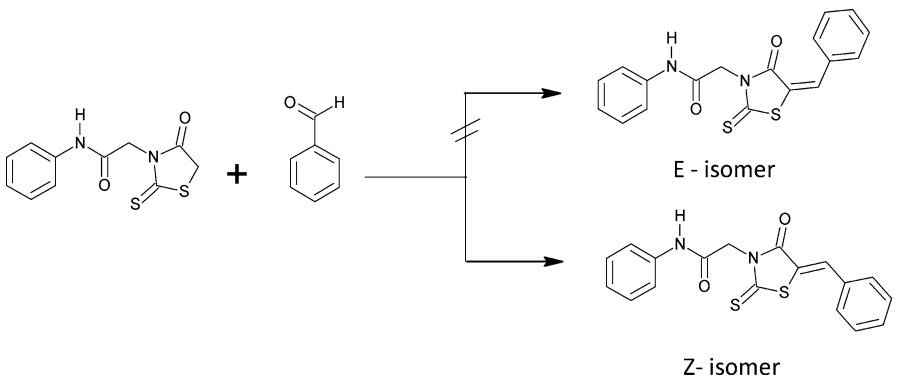


Fig. 2 *E–Z* isomerism of the synthesized compounds

observed in the range 129.12–120.04 ppm, and aliphatic carbon of the amide linkage at the N_3 position in the range 35.48–37.23 ppm. In the mass spectrum of compound **7a**, the most stable fragment, with intensity 100 %, was at *m*/*z* 323 and the molecular ion peak was at 359 *m*/*z* (intensity 10 %). The physical and analytical data of compounds **7a**–**o** are listed in Table 1.

Table 1 Physical and analytical data of 2-[(5*Z*)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-*N*-phenylacetamides **7a–o**

Entry	Mol. formula	Yield (%)	M.W.	M.P. (°C)	Elemental analysis					
					Found (%)			Calculated (%)		
					C	H	N	C	H	N
7a	$C_{18}H_{14}N_2O_2S_2$	78.09	354.45	261–263	61.03	3.94	7.88	60.99	3.98	7.90
7b	$C_{18}H_{14}ClN_2O_2S_2$	75.83	388.89	278–279	55.62	3.39	7.19	55.59	3.37	7.20
7c	$C_{18}H_{14}ClN_2O_2S_2$	76.19	388.89	259–260	55.61	3.38	7.18	55.59	3.37	7.20
7d	$C_{18}H_{14}ClN_2O_2S_2$	78.24	388.89	258–259	55.62	3.40	7.21	55.59	3.37	7.20
7e	$C_{18}H_{14}Cl_2N_2O_2S_2$	69.36	423.34	267–268	51.09	2.88	6.63	51.07	2.86	6.62
7f	$C_{18}H_{14}FN_2O_2S_2$	68.57	372.44	261–262	58.06	3.55	7.53	58.05	3.52	7.52
7g	$C_{18}H_{14}BrN_2O_2S_2$	70.49	433.34	>280	49.93	3.09	6.48	49.89	3.02	6.46
7h	$C_{19}H_{16}N_2O_2S_2$	72.44	368.47	249–250	61.98	4.41	7.63	61.93	4.38	7.60
7i	$C_{19}H_{16}N_2O_2S_2$	71.68	368.47	235–236	61.97	4.40	7.62	61.93	4.38	7.60
7j	$C_{19}H_{16}N_2O_3S_2$	66.92	384.47	277–278	59.37	4.21	7.29	59.35	4.19	7.29
7k	$C_{19}H_{16}N_2O_3S_2$	68.79	384.47	253–254	59.38	4.20	7.31	59.35	4.19	7.29
7l	$C_{20}H_{18}N_2O_4S_2$	65.32	414.50	263–234	57.96	4.42	6.77	57.95	4.38	6.76
7m	$C_{18}H_{14}N_3O_4S_2$	73.03	399.44	>280	54.13	3.30	10.51	54.12	3.28	10.52
7n	$C_{18}H_{14}N_3O_4S_2$	74.34	399.44	275–276	54.13	3.25	10.51	54.12	3.28	10.52
7o	$C_{19}H_{14}F_3N_2O_2S_2$	64.21	422.44	>280	54.05	3.12	6.62	54.02	3.10	6.63

Biological evaluation

Compounds **7a–o** were assayed for in vitro antimicrobial activity by using the serial broth dilution technique to determine MIC values and the Kirby Bauer disk diffusion technique to determine the zone of inhibition. Activity was determined against a variety of microorganisms (Eukaryotes and Prokaryotes), including bacteria (Gram +ve bacilli, *Bacillus subtilis*; Gram +ve cocci, *Staphylococcus aureus*; Gram –ve bacilli, *Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (yeast strain, *Candida albicans*; and filamentous fungi, *Aspergillus niger*) (Figs. 3 and 4).

Evaluation of antibacterial activity

The results obtained from testing of **7a–o** against selected Gram-positive and Gram-negative bacterial strains are reported in Table 2 (serial broth dilution technique to determine MIC) and Table 3 (Kirby Bauer technique to determine zone of inhibition); in the tables the results are compared with those obtained by use of reference drugs. From the activity results, the effects of the substituent at the 5-benzylidine ring of 4-thiazolidinones was evaluated. By merging the results obtained from both the techniques (MIC determination and zone of inhibition study), activity differences between compounds with the same MIC value were measured.

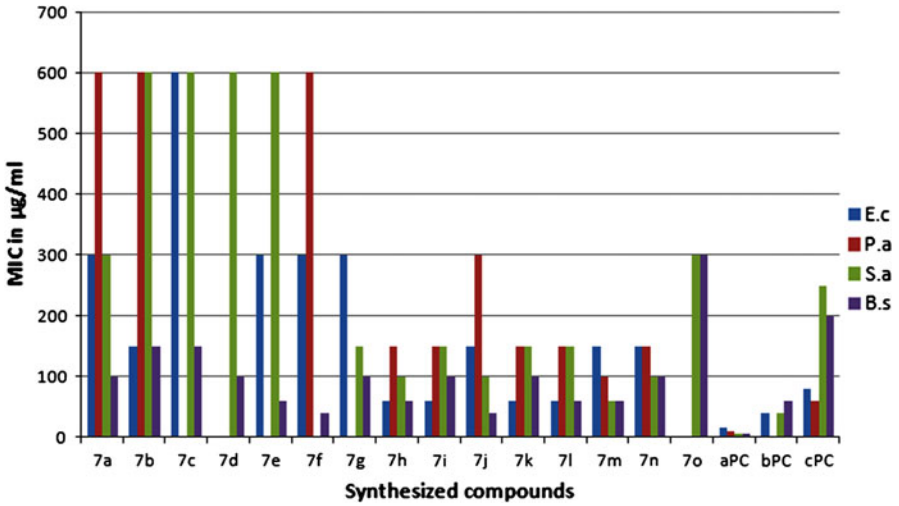


Fig. 3 Graphical representation of antibacterial activity of the synthesized compounds

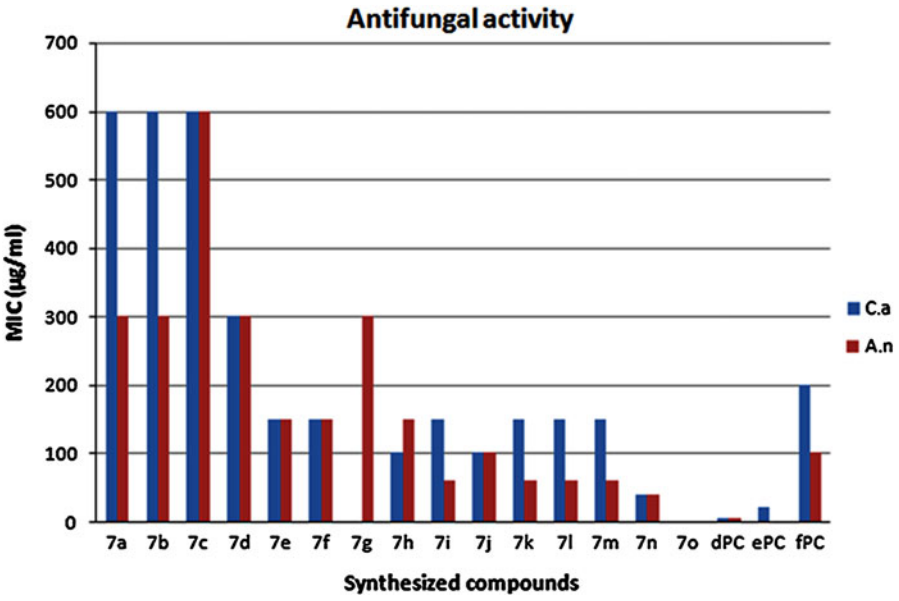


Fig. 4 Graphical representation of antifungal activity of the synthesized compounds

Compounds **7h**, **7i**, **7k**, and **7l** inhibited bacterial strain *E. coli* with an MIC of 60 µg/ml. Compounds with electron-releasing $-CH_3$ and $-OCH_3$ groups on the 5-benzylidine ring (**7h**, **7i**, **7k**, and **7l**) were more active against *E. coli* than compounds with electron-withdrawing –Cl, –F, –Br, $-NO_2$ and $-CF_3$ groups (**7b**, **7c**, **7d**, **7e**, **7f**, **7g**, **7m**, **7n**, and **7o**). Compounds with the electron-withdrawing $-NO_2$

Table 2 In-vitro antibacterial and antifungal activity (MIC determination by serial broth dilution technique) of 2-[(5*Z*)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-*N*-phenylacetamides **7a–o**

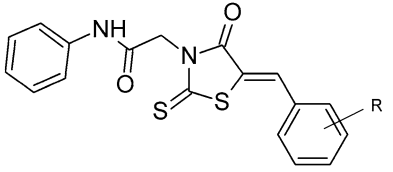
Synthesized compounds	R	log *p*	MIC (μg/ml)					
			Gram negative strains		Gram positive strains		Fungal strains	
			E.c	*P.a*	*S.a*	*B.s*	*C.a*	*A.n*
7a	H	2.79	300	600	300	100	600	300
7b	4-Cl	3.34	150	600	600	150	600	300
7c	3-Cl	3.34	600	>600	600	150	600	600
7d	2-Cl	3.34	>600	>600	600	100	300	300
7e	2,4-Cl	3.90	300	>600	600	60	150	150
7f	3-F	2.94	300	600	>600	40	150	150
7g	4-Br	3.61	300	>600	150	100	>600	300
7h	4-Me	3.27	60	150	100	60	100	150
7i	3-Me	3.27	60	150	150	100	150	60
7j	4-OCH_3	2.66	150	300	100	40	100	100
7k	3-OCH_3	2.66	60	150	150	100	150	60
7l	3,4-OCH_3	2.53	60	150	150	60	150	60
7m	4-NO_2	3.23	150	100	60	60	150	60
7n	3-NO_2	3.23	150	150	100	100	40	40
7o	4-CF_3	3.71	>600	>600	300	300	>600	>600
NC	DMSO	–	–	–	–	–	–	–
[a]PC	Ciprofloxacin	–	15	10	5	5	–	–
[b]PC	Ampicillin	–	40	>250	40	60	–	–
[c]PC	Carbenicillin	–	80	60	250	200	–	–
[d]PC	Flucanazole	–	–	–	–	–	5	5
[e]PC	Ketaconazole	–	–	–	–	–	20	>300
[f]PC	Itraconazole	–	–	–	–	–	200	100

log *p* value was calculated by use of Chem Draw ultra-7.0.1 software

E.c, *Escherichia coli*; P.a, *Pseudomonas aeruginosa*; S.a, *Staphylococcus aureus*; B.s, *Bacillus subtilis*; C.a, *Candida albicans*; NC, negative control (effect of DMSO on microbes); *PC*, positive control (standard reference drugs)

group on the 5-benzylidine ring (**7m** and **7n**) were more active against *E. coli* (MIC, 150 μg/ml) than compounds with electron-withdrawing groups –Cl, –F, –Br, and –CF_3 (**7c**, **7d**, **7e**, **7f**, **7g** and **7o**). Compounds **7h**, **7i**, **7k**, **7l**, and **7n** were more active against the Gram-negative strain *P. aeruginosa* (MIC 150 μg/ml) than the other compounds, which were poorly active. Substitution with electron-releasing –CH_3 and –OCH_3 groups on the 5-benzylidine ring (**7h**, **7i**, **7j**, **7k** and **7l**) increased inhibition potency compared with the unsubstituted parent compound **7a** (MIC, 600 μg/ml). In general, from the results obtained it was concluded that electron-releasing groups (–CH_3 and –OCH_3) on the 5-benzylidine ring favour inhibition of Gram-negative strain *P. aeruginosa* more than electron-withdrawing groups, with the exception of compounds having the –NO_2 group.

Table 3 In-vitro antibacterial and antifungal activity (determination of zone of inhibition by Kirby Bauer disk diffusion technique) of 2-[(5*Z*)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-*N*-phenylacetamides **7a–o**



Entry	*E.c*			*P.a*			*S.a*			*B.s*			*C.a*			*A.n*		
	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml	IC µg/ml	100 µg/ml ZD (mm)	150 µg/ml
7a	300	–	–	600	–	–	300	–	–	100	12	15	600	–	–	300	–	–
7b	150	–		600	–	–	600	–	–	150	–	11	600	–	–	300	–	–
7c	600	–	–	>600	–	–	600	–	–	150	–	12	600	–	–	600	–	–
7d	>600	–	–	>600	–	–	600	–	–	100	11	13	300	–	–	300	–	–
7e	300	–	–	>600	–	–	600	–	–	60	13	15	150	–	12	150	–	12
7f	300	–	–	600	–	–	>600	–	–	40	14	16	150	–	11	150	–	11
7g	300	–	–	>600	–	–	150	–	11	100	11	13	>600	–	–	300	–	–
7h	60	12	14	150	–	11	100	11	12	60	12	15	100	12	14	150	–	12
7i	60	13	15	150	–	12	150	–	11	100	11	13	150	–	11	60	12	15
7j	150	–	12	300	–	–	100	13	14	40	14	17	100	11	13	100	11	13
7k	60	13	15	150	–	11	150	–	13	100	11	12	150	–	12	60	12	16
7l	60	13	17	150	–	12	150	–	11	60	12	14	150	–	11	60	13	15
7m	150	–	11	150	–	11	60	13	17	60	12	15	150	–	12	60	12	14
7n	150	–	12	150	–	11	100	11	13	100	12	13	40	14	18	40	15	18
7o	>600	–	–	>600	–	–	300	–	–	300	–	–	>600	–	–	>600	–	–
NC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PC1	20	–	NT	10	–	NT	10	–	NT	05	–	NT	–	–	–	–	–	–
PC2	40	–	NT	>100	–	NT	40	–	NT	60	–	NT	–	–	–	–	–	–

Table 3 continued

Entry	*E.c*			*P.a*			*S.a*			*B.s*			*C.a*			*A.n*		
	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml	IC μg/ml	100 μg/ml ZD (mm)	150 μg/ml
PC3	80	–	NT	60	–	NT	>100	–	NT	>100	–	NT	–	–	–	–	–	–
PC4	–	–	–	–	–	–	–	–	–	–	–	–	5	–	NT	5	–	NT
PC5	–	–	–	–	–	–	–	–	–	–	–	–	20	–	NT	>300	–	NT
PC6	–	–	–	–	–	–	–	–	–	–	–	–	200	–	NT	100	–	NT

IC, initial concentration (maximum dilution) for appearance of zone; ZD, zone diameter in mm; E.c, *Escherichia coli*; P.a, *Pseudomonas aeruginosa*; S.a, *Staphylococcus aureus*; B.s, *Bacillus subtilis*; C.a, *Candida albicans*; NC, negative control (effect of DMSO on microbes); PC1–6, positive control (standard reference drugs); PC1, ciprofloxacin; PC2, ampicillin; PC3, carbenicillin; PC4, flucanazole; PC5, ketaconazole; PC6, itraconazole; NT, not tested

For strain *S. aureus*, it was observed that compounds with a halogen substituent on the 5-benzylidine ring (**7b**, **7c**, **7d**, **7e** and **7f**; MIC $\geq$600 μg/ml) were less active than the unsubstituted parent compound **7a** (MIC, 300 μg/ml), exception for compound **7g**. Compound **7m**, with the electron-withdrawing $-NO_2$ group at the *para* position of the 5-benzylidine ring was most active against *S. aureus* (MIC 60 μg/ml). Compounds with the substituents –F and $-OCH_3$ at the *meta* and *para* positions, respectively, of the 5-benzylidine ring had very good potency against *B. subtilis* (MIC 40 μg/ml) and compounds **7e**, **7h**, **7l**, and **7m** also had good inhibition potency (MIC 60 μg/ml). For inhibition of strain *B. subtilis,* no particular inhibition pattern was observed. Focussing on the overall activity profile against the strains tested, it was observed that the compounds had better inhibition potency against *B. subtilis* than against the other Gram-positive and Gram-negative bacterial strains (*E. coli*, *P. aeruginosa*, and *S. aureus*).

Evaluation of antifungal activity

The results obtained from antifungal testing of compounds **7a**–**o** against selected strains *C. albicans* and *A. niger* are reported in Table 2 (serial broth dilution technique to determine MIC) and Table 3 (Kirby Bauer technique to determine zone of inhibition); in the tables the results are compared with those obtained by use of reference drugs (Scheme 2).

The compound with the unsubstituted 5-benzylidine ring (**7a**) was inactive (MIC 600 μg/ml) against fungal strain *C. albicans*, but compounds with –F and $-NO_2$ substituents (**7f**, **7m**, and **7n**) were more active. Compound **7n**, with an $-NO_2$ group at the *meta* position was the most active compound (MIC 40 μg/ml). Electron-releasing methyl and methoxy groups at the *meta* and *para* positions of the 5-benzylidine ring, increased anticandida potency (**7a**, MIC 600 μg/ml; **7h**, **7i**, **7j**, **7k**, and **7l**, MIC $\leq$150 μg/ml). A somewhat similar trend was observed for inhibition of the fungal strain *A. niger*; compounds with an $-NO_2$ group on the 5-benzylidine ring (**7m**, **7n**) had comparatively high activity. Compound **7n** had highest antifungal activity against *A. niger* (MIC 40 μg/ml).

Experimental procedure

Materials and instrumentation

All reagents were of analytical reagent grade and were used without further purification. Solvents used were of analytical grade and used without further purification. 2-Thioxo-4-thiazolidinone was purchased from Sigma Aldrich Chemicals, Mumbai, India. 2-Chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 4-methylbenzaldehyde, and 3-methylbenzaldehyde were gifts from Benzo Chem Industries, Jalgaon, India.

Melting points were determined in open capillaries on a Veego model VMP-D electronic apparatus (Veego Instrument Corporation, Mumbai, India) and are uncorrected. To monitor the reactions and to establish the identity and purity of

reactants and products, thin-layer chromatography was performed on E. Merck silica gel plates with 0.50 mm layers and spots were visualized under UV radiation. FT-IR spectra (4,000–400 cm^{-1}) were recorded as KBr disks on a Shimadzu spectrophotometer (model: 8400-S; Shimadzu India, Mumbai, India). ^{1}H NMR and ^{13}C NMR spectroscopy was performed at CSMCRI, Bhavnagar, India, on a 500 MHz instrument, using $CDCl_3$ as solvent and TMS as internal reference (chemical shifts are in δ ppm). Mass analysis was performed at Oxygen Healthcare Research, Ahmedabad, India, by use of ESI–MS.

Synthesis of potassium 4-oxo-2-thioxo-1,3-thiazolidin-3-ide

The potassium salt of 2-thioxo-4-thiazolidinone was synthesized by use of a procedure reported elsewhere [20].

Synthesis of 2-(4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-N-phenylacetamide

The *N*-potassium salt of 2-thioxo-4-thiazolidinone (10 mmol, 1.713 g) was dissolved in 1:1 EtOH–DMF with 2-chloro-*N*-phenyl acetamide (11 mmol, 1.869 g). Catalytic amounts of potassium iodide and carbonate were added and the mixture was heated under reflux for approximately 5 h. The status of the reaction was monitored by TLC on silica gel with toluene–acetone 9:1 as mobile phase. After complete conversion, the solvent was removed by rotary evaporation under vacuum. The residue was triturated with *n*-hexane then treated with crushed ice. The product was then extracted with dichloromethane, followed by separation of the organic layer. The organic layer was treated with sodium sulfate then filtered. The filtrate was evaporated up to dryness under vacuum to obtain 2-(4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-*N*-phenylacetamide in the pure form which was recrystallized from EtOH–DMF or glacial acetic acid.

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.79 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.11–7.68 (5H, m, Ar–<u>H</u>), 3.45 (2H, s, –C<u>H</u>$_2$), 3.23 (2H, s, –C<u>H</u>$_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.10 (<u>C</u>=O), 137.21 (exocyclic =<u>C</u>H), 129.12–120.04 (Ar–<u>C</u>), 36.86 (–<u>C</u>H$_2$), 33.49 (–<u>C</u>H$_2$); IR (KBr, cm^{-1}): 3297.59 (–NH), 1691.08, 1695.13 (C=O str.), 1,158.16 (C–N str.)

General procedure for synthesis of 2-[(5Z)-5-benzylidene-4-oxo-2-thioxo-1, 3-thiazolidin-3-yl]-N-phenylacetamides

Compounds **7a–o** were synthesized by treating the intermediate 2-(4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-*N*-phenylacetamide with different benzaldehyde derivatives. First the benzaldehyde was dissolved in ethanol with an equimolar amount of piperidine as catalyst, followed by addition of equimolar amount of intermediate **5**. The reaction mixture was heated under reflux for approximately 5–9 h. The status of the reaction was monitored by TLC on silica gel with 5 % MeOH in $CHCl_3$ as mobile phase. After complete conversion, the reaction mixture was treated with crushed ice, filtered, and the residue was dried and recrystallized from ethanol to furnish the final compound.

The same Knoevenagel condensation was also performed in acidic medium, by dissolving the intermediate **5** in glacial acetic acid with 2.1 equiv anhydrous sodium acetate and an equimolar amount of benzaldehyde. The reaction mixture was heated under reflux for approximately 10–15 h then treated with crushed ice to separate the product. The product was isolated by filtration, washed, dried, and recrystallized from glacial acetic acid.

The Knoevenagel condensation reaction of intermediate **5** in both media suggested that reaction time and yield were better under basic conditions. So, for synthesis of the other compounds basic conditions were preferred.

*2-[(5Z)-5-benzylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7a**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.66 (1H, s, –NH, exchangeable with D_2O), 7.90 (1H, s, =CH exocyclic), 7.11–7.56 (10H, m, Ar–H), 3.45 (2H, s, –CH_2); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.10 (C=O), 137.28 (exocyclic =CH), 129.12–120.04 (Ar–C), 36.86 (–CH_2); IR (KBr, cm^{-1}): 3297.59 (–NH), 1,691.08, 1,695.13 (C=O str.), 1,158.16 (C–N str.); ESI–MS (*m*/*z*): 359 (10 %), 323 (100 %), 300 (92 %).

*2-[(5Z)-5-(4-chlorobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7b**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.72 (1H, s, –NH, exchangeable with D_2O), 7.92 (1H, s, =CH exocyclic), 7.07–7.58 (9H, m, Ar–H), 3.49 (2H, s, –CH_2); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 166.21 (C=O), 137.31 (exocyclic,=CH), 129.01–120.12 (Ar–C), 36.92 (–CH_2); IR (KBr, cm^{-1}): 3298.63 (–NH), 1,689.08, 1,698.13 (C=O str.), 1,158.73 (C–N str.), 693.23 (C–Cl str.); ESI–MS (*m*/*z*): 391 (10 %), 360 (100 %), 334 (89 %).

*2-[(5Z)-5-(3-chlorobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7c**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.78 (1H, s, –NH, exchangeable with D_2O), 7.92 (1H, s, =CH exocyclic), 7.12–7.60 (9H, m, Ar–H), 3.54 (2H, s, –CH_2); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 166.23 (C=O), 137.29 (exocyclic =CH), 129.47–121.34 (Ar–C), 37.12 (–CH_2); IR (KBr, cm^{-1}): 3,310.63 (–NH str.), 1,689.08–1,699.24 (C=O str.), 1,151.33 (C–N str.), 692.22 (C–Cl str.); ESI–MS (*m*/*z*): 392 (9 %), 361 (100 %), 334 (89 %).

*2-[(5Z)-5-(2-chlorobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7d**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.76 (1H, s, –NH, exchangeable with D_2O), 7.97 (1H, s, =CH exocyclic), 7.14–7.55 (9H, m, Ar–H), 3.44 (2H, s, –CH_2); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 166.31 (C=O), 136.93 (exocyclic =CH), 128.85–119.94 (Ar–C), 36.94 (–CH_2); IR (KBr, cm^{-1}): 3295.49 (–NH), 1,690.16–1,698.13 (C=O str.), 1,157.36 (C–N str.), 696.14 (C–Cl str.); ESI–MS (*m*/*z*): 391 (9 %), 360 (100 %), 334 (90 %).

*2-[(5Z)-5-(2,4-dichlorobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7e**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.72 (1H, s, –NH, exchangeable with D_2O), 7.96 (1H, s, =CH exocyclic), 7.11–7.63 (8H, m, Ar–H),

3.44 (2H, s, –C<u>H</u>$_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 165.98 (<u>C</u>=O), 136.91 (exocyclic =<u>C</u>H), 129.13–120.49 (Ar–<u>C</u>), 37.02 (–<u>C</u>H$_2$); IR (KBr, cm^{-1}): 3307.46 (–NH), 1,691.16, 1,696.79 (C=O str.), 1161.69 (C–N str.), 692.19 (C–Cl str.); ESI–MS (*m*/*z*): 427 (8 %), 394 (100 %), 368 (89 %).

*2-[(5Z)-5-(3-fluorobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7f**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.75 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.95 (1H, s, =C<u>H</u> exocyclic), 7.11–7.62 (9H, m, Ar–<u>H</u>), 3.48 (2H, s, –C<u>H</u>$_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 166.18 (<u>C</u>=O), 137.29 (exocyclic =<u>C</u>H), 128.57–121.16 (Ar–<u>C</u>), 36.99 (–<u>C</u>H$_2$); IR (KBr, cm^{-1}): 3,288.52 (–NH), 1,686.49–1,697.37 (C=O str.), 1,289.19 (C–F str.), 1,156.19 (C–N str.); ESI–MS (*m*/*z*): 375 (9 %), 343 (100 %), 317 (87 %).

*2-[(5Z)-5-(4-bromobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7g**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.81 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.93 (1H, s, =C<u>H</u> exocyclic), 7.19–7.78 (9H, m, Ar–<u>H</u>), 3.53 (2H, s, –C<u>H</u>$_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.22 (<u>C</u>=O), 138.88 (exocyclic =<u>C</u>H), 129.64–120.37 (Ar–<u>C</u>), 36.79 (–<u>C</u>H$_2$); IR (KBr, cm^{-1}): 3,311.63 (–NH), 1,677.08, 1687.22 (C=O str.), 1,164.49 (C–N str.); ESI–MS (*m*/*z*): 437 (9 %), 404 (100 %), 377 (86 %).

*2-[(5Z)-5-(4-methylbenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7h**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.65 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.93 (1H, s, =C<u>H</u> exocyclic), 7.03–7.79 (9H, m, Ar–<u>H</u>), 3.45 (2H, s, –C<u>H</u>$_2$), 2.44 (3H, s, –C<u>H</u>$_3$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.52 (<u>C</u>=O), 136.61 (exocyclic =<u>C</u>H), 129.09–120.22 (Ar–<u>C</u>), 36.07 (–<u>C</u>H$_2$), 21.23 (–<u>C</u>H$_3$); IR (KBr, cm^{-1}): 3,289.41 (–NH), 1,688.47, 1,697.12 (C=O str.), 1,163.43 (C–N str.); ESI–MS (*m*/*z*): 372 (10 %), 339 (100 %), 313 (88 %).

*2-[(5Z)-5-(3-methylbenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7i**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.69 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.97 (1H, s, =C<u>H</u> exocyclic), 7.14–7.71 (9H, m, Ar–<u>H</u>), 3.46 (2H, s, –C<u>H</u>$_2$), 2.49 (3H, s, –C<u>H</u>$_3$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.83 (<u>C</u>=O), 136.88 (exocyclic =<u>C</u>H), 129.13–121.49 (Ar–<u>C</u>), 36.33 (–<u>C</u>H$_2$), 21.49 (–<u>C</u>H$_3$); IR (KBr, cm^{-1}): 3,297.76 (–NH), 1,676.39, 1,697.25 (C=O str.), 1,167.64 (C–N str.); ESI–MS (*m*/*z*): 371 (9 %), 340 (100 %), 313 (89 %).

*2-[(5Z)-5-(4-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7j**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.71 (1H, s, –N<u>H</u>, exchangeable with D_2O), 7.96 (1H, s, =C<u>H</u> exocyclic), 7.13–7.85 (9H, m, Ar–<u>H</u>), 3.54 (3H, s, –OC<u>H</u>$_3$), 3.39 (2H, s, –C<u>H</u>$_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.10 (<u>C</u>=O), 154.36 (Ar<u>C</u>–OCH$_3$), 137.28 (exocyclic =<u>C</u>H), 129.12–120.04 (Ar–<u>C</u>), 56.82 (–O<u>C</u>H$_3$), 36.86 (–<u>C</u>H$_2$); IR (KBr, cm^{-1}): 3,297.59 (–NH), 1,691.08, 1,695.13 (C=O str.), 1,158.16 (C–N str.); ESI–MS (*m*/*z*): 388 (10 %), 355 (100 %), 328 (89 %).

*2-[(5Z)-5-(3-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7k**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.77 (1H, s, –NH, exchangeable with D_2O), 7.96 (1H, s, =CH exocyclic), 7.09–7.72 (9H, m, Ar–H), 3.41 (3H, s, $-OCH_3$), 3.28 (2H, s, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.23 (C=O), 151.38 (ArC–OCH_3), 137.25 (exocyclic =CH), 128.23–120.09 (Ar–C), 55.39 ($-OCH_3$), 36.55 ($-CH_2$); IR (KBr, cm^{-1}): 3,309.47 (–NH), 1,689.08, 1,701.34 (C=O str.), 1,160.16 (C–N str.); ESI–MS (*m*/*z*): 388 (9 %), 354 (100 %), 329 (85 %).

*2-[(5Z)-5-(3,4-dimethoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7l**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.73 (1H, s, –NH, exchangeable with D_2O), 7.98 (1H, s, =CH exocyclic), 7.31–7.59 (8H, m, Ar–H), 3.61 (6H, s, $-OCH_3$), 3.25 (2H, s, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 168.97 (C=O), 149.22 (ArC-OCH_3), 135.11 (exocyclic =CH), 129.32–120.63 (Ar–C), 54.84 ($-OCH_3$), 36.54 ($-CH_2$); IR (KBr, cm^{-1}): 3,313.68 (–NH), 1,678.08, 1,701.34 (C=O str.), 1,163.52 (C–N str.); ESI–MS (*m*/*z*): 418 (9 %), 385 (100 %), 359 (88 %).

*2-[(5Z)-5-(4-nitrobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7m**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.10 (1H, s, Ar–H, adjacent to $-NO_2$ group), 9.09 (1H, d, Ar–H, adjacent to $-NO_2$ group), 8.63 (1H, s, –NH, exchangeable with D_2O), 7.94 (1H, s, =CH exocyclic), 7.08–7.65 (7H, m, Ar–H), 3.47 (2H, s, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 167.52 (C=O), 136.33 (exocyclic =CH), 129.13–120.69 (Ar–C), 37.06 ($-CH_2$); IR (KBr, cm^{-1}): 3,301.53 (–NH), 1,660.48, 1695.13 (C=O str.), 1,154.31 (C–N str.); ESI–MS (*m*/*z*): 403 (10 %), 370 (100 %), 343 (89 %).

*2-[(5Z)-5-(3-nitrobenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (**7n**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.93 (1H, s, Ar–H, adjacent to $-NO_2$ group), 8.91 (1H, d, Ar–H, adjacent to $-NO_2$ group), 8.71 (1H, s, –NH, exchangeable with D_2O), 7.93 (1H, s, =CH exocyclic), 6.09–7.82 (7H, m, Ar–H), 3.45 (2H, s, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 168.52 (C=O), 135.33 (exocyclic =CH), 128.77–120.37 (Ar–C), 37.23 ($-CH_2$); IR (KBr, cm^{-1}): 3,316.15 (–NH), 1,661.67, 1,694.94 (C=O str.), 1,155.12 (C–N str.); ESI–MS (*m*/*z*): 402 (9 %), 371 (100 %), 344 (88 %).

*2-{(5Z)-4-oxo-2-thioxo-5-[4-(trifluoromethyl)benzylidene]-1,3-thiazolidin-3-yl}-N-phenylacetamide (**7o**)* ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.53 (1H, s, –NH, exchangeable with D_2O), 7.96 (1H, s, =CH exocyclic), 7.12–7.72 (9H, m, Ar–H), 3.51 (2H, s, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 168.64 (C=O), 139.34 (exocyclic =CH), 130.46 (Ar–C–CF_3), 128.37–121.16 (Ar–C), 119.61 ($-CF_3$), 35.48 ($-CH_2$); IR (KBr, cm^{-1}): 3,340.94 (–NH), 1,679.67, 1,698.16 (C=O str.), 1164.39 (C–N str.); ESI–MS (*m*/*z*): 427 (9 %), 393 (100 %), 367 (87 %).

Pharmacology

The newly synthesized compounds **7a–o** were evaluated for in-vitro antibacterial and antifungal activity. The standard strains used for antimicrobial activity were procured from Promotech Life sciences, Bangalore, India. In-vitro antibacterial activity was determined against Gram-positive bacterial strains *B. subtilis* ATCC 11774 and *S. aureus* ATCC 25923 and Gram-negative bacterial strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 by use of the Kirby Bauer technique and the serial broth dilution technique. Antifungal screening was determined against strains *C. albicans* ATCC 66027 and *A. niger* ATCC 6275 by the same method.

Kirby Bauer disk diffusion technique

The Kirby Bauer technique uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. Mueller–Hinton agar medium was sterilized (autoclaved at 120 °C for 30 min) then poured into a Petri dish to a uniform depth of 5 mm and left to solidify. The microbial suspension (10^5 CFU/ml) (0.5 McFarland nephelometry standards) was streaked over the surface of the agar, by use of a sterile cotton swab, for growth of the organisms. All the compounds (**7a–o**) were dissolved in dimethyl sulfoxide and seven dilutions of each compound (20, 40, 60, 100, 150, 300, and 600 μg/ml) were prepared and applied to wafers to measure activity. Sterile filter paper disks measuring 10 mm in diameter, previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide, were placed on an agar medium that had been inoculated with the respective microorganism and the resulting Petri dishes were incubated for 24 h at 37 °C [24].

In the biological evaluation DMSO was used as a negative control to check the effect of DMSO on the microbes. When a control disk impregnated with dimethyl sulfoxide without any sample was also used in the same manner no inhibition was observed. Standard antibacterial drugs ciprofloxacin, ampicillin, and carbenicillin were used as a reference drugs during antibacterial screening. Standard antifungal drugs flucanazole, ketaconazole, and itraconazole were used as a reference drugs during antifungal screening.

Serial broth dilution technique

MIC values of **7a–o** were determined by use of the serial broth dilution technique [25, 26] using a set of sterilized test tubes containing nutrient broth and capped with cotton plugs. Compounds **7a–o** were dissolved in DMSO and seven dilutions (20, 40, 60, 100, 150, 300, and 600 μg/ml) of each compound were prepared. Fixed volumes of culture and diluted compound solution were placed in each test tube and incubated at 37 °C for 24 h. The lowest concentration (highest dilution) resulting in no microbial growth was regarded as the MIC. The test mixture should contain 10^8 cells/ml. MIC values of the standard antibacterial drugs ciprofloxacin, ampicillin, and carbenicillin were also determined by the same procedure, in DMSO as solvent, to obtain reference results. Similarly, MIC values of the standard

antifungal drugs fluconazole, ketoconazole, and itraconazole, obtained by the same method, were regarded as a reference results.

Conclusion

From the bioactivity results it is clear that appropriate substituents on the 5-benzylidine ring lead to more active antimicrobial agents. It can also be stated that antibacterial and antifungal activity may be associated with the nature of the micro organisms tested and the chemical structures of the compounds tested. In general, it was concluded that compounds **7a–o** had better antibacterial activity against *B. subtilis* than against the other bacterial strains. Compounds with electron-releasing groups on the 5-benzylidine ring were more active than those bearing electron-withdrawing groups. Compounds with the $-NO_2$ substituent on the 5-benzylidine ring were more active than those bearing other electron-withdrawing groups. From the results obtained, more extensive study is also justified to gain deeper insight into structure–activity relationships, to obtain these types of structure which are more effective.

Acknowledgments The authors wish to thank the Department of Applied Chemistry, S.V. National Institute of Technology, Surat, India, for providing laboratory facilities and scholarship. The authors wish to offer their deep gratitude to Mr Paresh Kapopara, Central and Kashiba Diagnostic Laboratory and Microbial Research Centre, Surat, for performing biological screening. The authors are also grateful to the director, Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar, for performing spectral analysis and to Oxygen Healthcare Research Pvt. Ltd., Ahmedabad for performing mass analysis.

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