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Original article

Synthesis and antibacterial activity of 2-(4-substituted phenyl)-3(2H)-isothiazolones

Ali Khalaj [a,*], Neda Adibpour [a], Ahmad Reza Shahverdi [b], Mohsen Daneshtalab [c]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran Medical Sciences University, P.O. Box 14155/6451, Tehran, Iran*
[b] *Department of Biotechnology, Faculty of Pharmacy, Tehran Medical Sciences University, P.O. Box 14155/6451, Tehran, Iran*
[c] *School of Pharmacy, Memorial University of Newfoundland, Health Sciences Centre, 300 Prince Philip Drive, St. John's, Nfld, Canada A1B 3V6*

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Abstract

Several new and known 2-(4-substituted phenyl)-3(2H)-isothiazolone derivatives with or without chloro substituent at C-5 position were synthesized and their in vitro antibacterial activity against selected Gram-negative and Gram-positive bacteria were evaluated using agar dilution method. Most of compounds exhibited moderate to high activities against tested microorganisms, and in comparison with the reference drugs some compounds showed comparable or higher activities. In contrast to results of the previous studies, some 5-chloro derivatives showed lower or comparable activities against some tested microorganism, in comparison with analogues without C-5 substitution. In general, most of the compounds bearing electron withdrawing group at 4-position of the phenyl ring were more active against Gram-positive and most of those having piperazine derivatives were more active against Gram-negative bacteria.

Keywords: 3(2H)-Isothiazolones; *N*,*N*′-Dithiodipropionamides; Antibacterial activity; Hydrophobicity; Physicochemical properties

1. Introduction

The 3(2H)-isothiazolone derivatives have shown antibacterial, antifungal, pesticidal and algaecidal activities and have shown to be useful as preservatives for the control of living organisms in cosmetics, paints, soaps, fabrics, leather, swimming pools and etc. [1]. In addition some derivatives have been reported to inhibit telomerase a novel and highly selective target for design of antitumor drugs [2] and to inhibit cartilage breakdown in arthritis by blockade of a matrix metalloproteinase [3].

The biological activity of these compounds is speculated to originate from their interaction with intracellular sulphur containing proteins, enzymes, or simple molecules such as glutathione leading to ring opening and disulfide bond formation and as a result impairment of the cell functions [4].

While several patents [5–8] of 3(2H)-isothiazolones with varying biocidal activities have been registered, the structure–activity relationship of these compounds have not been thoroughly investigated. From theoretical studies of a few number of 3(2H)-isothiazolones it has been concluded that structural effects do not likely play an important role in their mechanism of action but determine the reactivity of these compounds toward nucleophiles which result in ring opening and observed biological activity [9]. In an effort to elucidate the underlying structural requirement for the activity of 3(2H)-isothiazolones further, several *N*-phenyl isothiazolones substituted at the 4-position of the phenyl moiety with groups different in hydrophobicity, size, steric and electronic parameters were prepared. Synthesis of some of these compounds (**5a–c, 5e–g, 6a, 6e, 6g, 6l** and **6m**) has previously been reported but their physicochemical data (**6a**), spectral characteristics (**5a, 5b, 5f, 6a**) and biological data (**5f–g, 6a, 6g** and **6l**) were not generally available. This article describes synthesis, physicochemical properties and antibacterial activity of 3(2H)-isothiazolones **5a–j, 5s** and **6a–r** in comparison with ciprofloxacin, ceftriaxone, ceftazidime and gentamycin as reference drugs.

* Corresponding author. Tel.: +98-21-8962007; fax: +98-21-6461178.
E-mail address: khalaj@ams.ac.ir (A. Khalaj).

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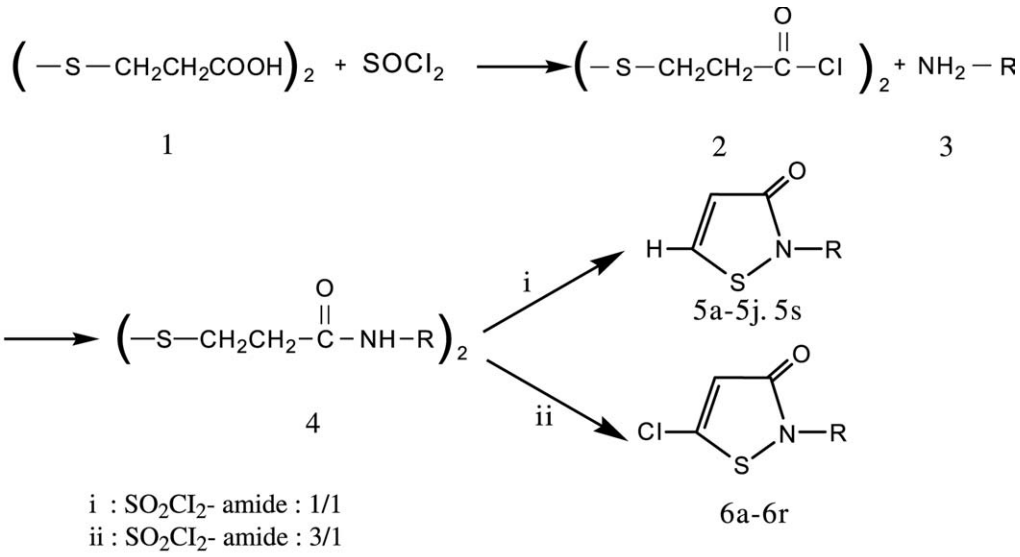
2. Chemistry

The 3(2H)-isothiazolones used in the present study were prepared by incremental addition of dichloromethane solution of sulfuryl chloride as an oxidizing agent to the solution of dithiodipropionamides in the same solvent at 0–10 °C [11]. By manipulation of the reaction stoichiometry, it was found that 5-chloro isothiazolone derivatives were the predominant products when the ratio of sulfuryl chloride/amide was 3:1 while with the relevant ratio of 1:1 the 5-unsubstituted analogues were the predominant products (Scheme 1).

All the synthesized 3(2H)-isothiazolones **5a–j, 5s** and **6a–r** were characterized by IR, ^{1}H-NMR and mass spectra data and C,H,N analyses and results are summarized in Tables 1 and 2. For the synthesis of dithiodipropionamides **4a–s**, dithiodipropionyl chlorides **2**, prepared from the reaction of the commercially available dithiodipropionic acid **1** with thionyl chloride were reacted with amines **3a–s**. Dithiodipropionamides **4a–c, 4e, 4l** and **4m** were prepared under the same reaction conditions as described earlier [11]. For the synthesis of **4a–e, 4k** and **4n–s**, dichloromethane and 25% aqueous sodium hydroxide solution and for the preparation of **4f–j, 4l** and **4m**, dichloromethane were employed as solvent. In all experiments dithiodipropionamides were precipitated from the reaction mixtures and purified by several washings with solvents of the reactions. The structures of the dithiodipropionamides **4a–s** were confirmed by IR, mass spectra data and (C, H, N) analyses and results are given in Tables 3 and 4.

3. In vitro antibacterial screening

The in vitro antibacterial activities of the synthesized compounds and reference drugs against Gram-positive (*Sta-*



Compound	R	Compound	R
a	Cl	k	$OOOCH_3$
b	NO_2	l	H
c	$COOC_2H_5$	m	H_2C
d	CF_3	n	
e		o	
f	CH_3	p	
g	OCH_3	q	$COOC_2H_5$
h	OCH_3CH_3	r	$CONH_2$
i	$OCH_2CH_2CH_3$	s	SCH_3
j	$N(CH_3)_2$		

Scheme 1. Synthesis of 2-(4-substituted phenyl)-3(2H)-isothiazolones.

Table 1
Yields and physicochemical compound properties of 3(2H)-isothiazolones **5a–j, 5s** and **6a–r** prepared

Compound	Yield (%)	M.p. (°C) (crystallization solvent)	Formula
5a	68.7	142–144 [a] (toluene)	
5b	71.0	188 [b] (EtOH)	
5c	19.6	142–144 [c] (toluene)	
5d	38.0	133–136 (EtOH-hexane)	$C_{10}H_6F_3NOS$
5e	37.0	90–91 [d] (toluene)	
5f	57.6	107 [e]	
5g	65.0	92–94 [f] (toluene)	
5h	43.6	98–101 (EtOAc–hexane)	$C_{11}H_{11}NO_2S$
5i	16.4	97 (EtOAc–hexane)	$C_{12}H_{13}N_2OS$
5j	16.5	90–91 (EtOAc)	$C_{11}H_{12}N_2OS$
5s	17.0	106–110 (EtOAc–hexane)	$C_{10}H_9NOS_2$
6a	59.6	128 (hexane)	$C_9H_5Cl_2NOS$
6b	14.6	175–178 (EtOH–petroleum ether)	$C_9H_5ClN_2O_3S$
6c	23.5	119–120 (toluene)	$C_{12}H_{10}ClNO_3S$
6d	47.6	86–87 (EtOH)	$C_{10}H_5ClF_3NOS$
6e	20.0	118 [g] (MeOH)	
6f	28.0	115–116 (hexane)	$C_{10}H_8ClNOS$
6g	55.0	105–108 [h]	
6h	18.0	96–100 (EtOAc)	$C_{11}H_{10}ClNO_2S$
6i	41.0	113–115 (EtOAc)	$C_{12}H_{12}ClNO_2S$
6j	21.5	87–90 (MeOH–hexane)	$C_{11}H_{11}ClNO_2S$
6k	34.0	135–137 (EtOAc)	$C_{11}H_8ClNO_3S$
6l	8.5	96–97 [I] (H_2O)	
6m	29.7	58 [j] (heptane)	
6n	33.7	173–174 (EtOAc–petroleum ether)	$C_{11}H_7ClN_4O_2S$
6o	5.2	125–128 (EtOH)	$C_{13}H_{14}ClN_3OS$
6p	25.0	134–138 (hexane)	$C_{19}H_{18}ClN_3OS$
6q	53.0	153–159 (hexane)	$C_{16}H_{18}ClN_3O_3S$
6r	26.0	138–143 (EtOAc)	$C_{14}H_{15}ClN_4O_2S$

[a] Ref. [11] gives 142–144 °C.
[b] Ref. [11] gives 188–190 °C.
[c] Ref. [11] gives 141–142 °C.
[d] Ref. [11] gives 91–92 °C.
[e] Ref. [21] gives 105–107 °C.
[f] Ref. [22] gives 92–93 °C.
[g] Ref. [23] gives 118–120 °C.
[h] Ref. [24] gives 105 °C.
[i] Ref. [11] gives 95–96 °C.
[j] Ref. [11] gives 58–59 °C.

phylococcus aureus ATCC 29737 (American type culture collection), *Staphylococcus epidermidis* ATCC 12229, *Bacillus subtilis* ATCC 12711) and Gram-negative (*Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 19430 and *Pseudomonas aeruginosa* ATCC 9027) bacteria were tested by the conventional agar dilution method [10] using Mueller Hinton agar medium and compared with those of ciprofloxacin, gentamycin, ceftazidime, ceftriaxone which were chosen as the reference drugs since they have shown an inhibitory effect on all bacteria used in this study [19]. Suspensions of each of bacteria were prepared to contain approximately 10^6 colony forming units (CFU/ml) and applied to plates with twofold serially diluted compounds to be tested in DMSO and incubated at 37 °C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilution as used in the experiments. The MIC values obtained for 3(2H)-isothiazolones **5a–j, 5s** and **6a–r** and reference drugs are reported in Table 5.

4. Results and discussion

The majority of the synthesized compounds displayed moderate–high activity against tested bacteria and had MICs in the range of the most active reference drugs. In particular compared with ciprofloxacin the most active reference drug against *S. aureus* (MIC 0.5 μg/ml) and *P. aeruginosa* (MIC 0.01 μg/ml). Compounds **6a** and **6m** showed higher in vitro potency against *S. aureus* (MIC 0.3 μg/ml) and compounds **6f, 6g, 6k** showed equal activity against *P. aeruginosa* (MIC 0.01 μg/ml).

Table 2
Spectroscopic data of 3(2H)-isothiazolones **5a–j, 5s** and **6a–r**

Compound	^{1}H NMR (δ)	IR (cm^{-1})	Mass m/e EI (relative abundance %)
5a	($CDCl_3$): 6.32 (d, 1H, CH, J = 4.0 Hz) 7.25–7.60 (m, 4H, Ar–H) 8.17 (d, 1H, CH, J = 4.0 Hz)	1660 (CO, amide)	211 (20 M^+), 177 (100)
5b	($CDCl_3$): 6.38 (d, 1H, CH, J = 4.0 Hz) 7.8 (d, 2H, Ar–H, J = 8.0 Hz) 8.30 (d, 2H, Ar–H, J = 8.0 Hz) 8.38 (d, 1H, CH, J = 4.0 Hz)	1671 (CO, amide); 1520, 1342 (NO_2)	222 (100 M^+), 176 (10)
5c [a]	($CDCl_3$): 1.41 (t, 3H, CH_3, J = 8.0 Hz) 4.40 (q, 2H, CH_2, J = 8.0 Hz) 6.35(d, 1H, CH, J = 4.0 Hz) 7.75 (d, 2H, Ar–H, J = 8.0 Hz) 8.12 (d, 2H, Ar–H, J = 8.0 Hz) 8.20 (d, 1H, CH, J = 4.0 Hz)	1723 (CO, ester); 1664 (CO, amide)	249 (90 M^+), 221 (35), 204 (100), 176 (30)
5d	($CDCl_3$): 6.33 (d, 1H, CH, J = 6.4 Hz) 7.73 (s, 4H, Ar–H) 8.19 (d, 1H, CH, J = 6.4 Hz)	1675 (CO, amide)	245 (100 M^+)
5e [b]	($CDCl_3$): 6.38 (d, 1H, CH, J = 6.4 Hz) 7.20–7.50 (m, 5H, Ar–H) 8.20 (d, 1H, CH, J = 6.4 Hz)	1656 (CO, amide)	177 (100 M^+), 99 (45)
5f	($CDCl_3$): 2.30 (s, 3H, CH_3) 6.31(d, 1H, CH, J = 6.4 Hz) 7.25 (d, 2H, Ar–H, J = 8.0 Hz) 7.43 (d, 2H, Ar–H, J = 8.0 Hz) 8.14 (d, 1H, CH, J = 8.0 Hz)	1654 (CO, amide)	191 (100 M^+), 176 (25)
5h	($CDCl_3$): 1.40 (t, 3H, CH_3, J = 8.1 Hz) 4.02 (q, 2H, CH_2, J = 8.1 Hz) 6.26 (d, 1H, CH, J = 6.4 Hz) 6.95 (d, 2H, Ar–H, J = 8.0 Hz) 7.44 (d, 2H, Ar–H, J = 8.0 Hz) 8.14 (d, 1H, CH, J = 6.4 Hz)	1656 (CO, amide); 1248 (CO, ether)	221 (100 M^+), 192 (80)
5i	($CDCl_3$): 1.02 (t, 3H, CH_3, J = 7.2 Hz) 1.75 (m, 2H, CH_2) 3.84 (t, 2H, OCH_2, J = 7.2 Hz) 6.28 (d, 1H, CH, J = 6.4 Hz), 6.90 (d, 2H, Ar–H, J = 8.0 Hz) 7.40 (d, 2H, Ar-H, J = 8.0 Hz) 9.14 (d, 1H, CH, J = 6.4 Hz)	1669 (CO, amide); 1246 (CO, ether)	235 (100 M^+), 192 (100)
5j	($CDCl_3$): 2.82 (s, 6H, $2NCH_3$) 6.30 (d, 1H, CH, J = 6.4 Hz) 7.02–7.52 (m, 4H, Ar–H) 8.18 (d, 1H, CH, J = 6.4 Hz)	1654 (CO, amide)	220 (100 M^+), 191 (11), 177 (27)
5s	($CDCl_3$): 6.29 (d, 1H, CH, J = 6.4 Hz) 7.27 (d, 2H, Ar–H, J = 8.0 Hz) 7.47 (d, 2H, Ar–H, J = 8.0 Hz) 9.26 (d, 1H, CH, J = 6.4 Hz)	1644 (CO, amide)	223 (100 M^+), 208 (37)
6a	($CDCl_3$): 6.50 (s, 1H, CH) 7.40 (d, 2H, Ar–H, J = 8.0 Hz) 7.55 (d, 2H, Ar–H, J = 8.0 Hz)	1666 (CO, amide)	248 (8 M+2), 246 (20 M^+), 211 (100)
6b	($CDCl_3$): 6.40 (s, 1H, CH) 7.81 (d, 2H, Ar–H, J = 8.0 Hz) 8.30 (d, 2H, Ar–H, J = 8.0 Hz)	1690 (CO, amide); 1516, 1301 (NO_2)	258 (41 M+2), 256 (100 M^+), 210 (10)
6c	($CDCl_3$): 1.40 (t, 3H, CH_3, J = 8.1 Hz) 4.40 (q, 2H, CH_2, J = 8.1 Hz) 6.37 (s, 1H, CH) 7.65 (d, 2H, Ar–H, J = 8.0 Hz) 8.15 (d, 2H, Ar–H, J = 8.0 Hz)	1725 (CO, ester); 1667 (CO, amide)	285 (45 M+2), 283 (100 M^+), 255 (35), 238 (100), 210 (18)
6d	($CDCl_3$): 6.30 (s, 1H, CH) 7.70 (s, 4H, Ar–H)	1678 (CO, amide)	281 (36 M+2), 279 (100 M^+), 244 (23)
6e [c]	($CDCl_3$): 6.30 (s, 1H, CH) 7.30–7.60 (m, 5H, Ar–H)	1659 (CO, amide)	213 (43 M+2), 211 (100 M^+), 99 (56)
6f	($CDCl_3$): 2.37 (s, 3H, CH_3) 6.35 (s, 1H, CH) 7.25 (d, 2H, Ar–H, J = 8.0 Hz) 7.36 (d, 2H, Ar–H, J = 8.0 Hz)	1661 (CO, amide)	227 (43 M+2), 225 (100 M^+), 210 (10), 190 (72)
6g	($CDCl_3$): 3.90 (s, 3H, CH_3) 6.34 (s, 1H, CH) 6.90 (d, 2H, Ar–H, J = 8.0 Hz) 7.45 (d, 2H, Ar–H, J = 8.0)	1660 (CO, amide); 1251 (CO, ether)	243 (45 M+2), 241(100 M^+), 226 (10), 190 (10)
6h	($CDCl_3$): 1.41 (t, 3H, CH_3, J = 8.1 Hz) 4.01(q, 2H, CH_2, J = 8.1 Hz) 6.33 (s, 1H, CH) 6.90 (d, 2H, Ar–H, J = 8.0 Hz) 7.37 (d, 2H, Ar–H, J = 8.0 Hz)	1661 (CO, amide); 1254 (CO, ether)	257 (45 M+2), 255 (100 M^+), 227 (42)
6i	($CDCl_3$): 1.03 (t, 3H, CH_3, J = 7.2 Hz) 1.68–1.94 (m, 2H, CH_2) 3.92 (t, 2H, OCH_2, J = 7.2 Hz) 6.31 (s, 1H, CH) 6.85 (d, 2H, Ar–H, J = 8.0 Hz) 7.36 (d, 2H, Ar–H, J = 8.0 Hz)	1671 (CO, amide); 1246 (CO, ether)	271 (50 M+2), 269 (100 M^+), 226 (100), 192 (30)
6j	($CDCl_3$): 2.83 (s, 6H, $2NCH_3$) 6.34 (s, 1H, CH) 7.00–7.50 (m, 4H, Ar–H)	1659 (CO, amide)	256 (45 M+2), 254 (100 M^+)
6k	($CDCl_3$): 2.31 (s, 3H, CH_3) 6.36 (s, 1H, CH) 7.17 (d, 2H, Ar–H, J = 8.0 Hz) 7.53 (d, 2H, Ar–H, J = 8.0 Hz)	1760 (CO, ester); 1678 (CO, amide)	271 (5 M+2), 269 (10 M^+), 254 (10), 226 (35), 220 (100), 191(70)
6l [b]	($CDCl_3$): 6.53 (s, 1H, CH) 11.2 (broad, 1H, NH)	3449 (OH); 1643 (CO, amide)	137 (41 M+2), 135 (100 M^+)
6m [b]	($CDCl_3$): 4.86 (s, 2H, CH_2) 6.25 (s, 1H, CH) 7.31 (s, 5H, Ar–H)	1662 (CO, amide)	227 (40 M+2), 225 (100 M^+)
6n	($CDCl_3$): 6.26 (s, 1H, CH) 6.73 (d, 2H, Ar–H, J = 8.1 Hz) 7.18 (d, 2H, Ar–H, J = 8.1 Hz) 8.06 (s, 1H, CH) 11.70 (broad, 1H, NH)	3349 (NH, amide); 1705, 1686 (CO, amide)	296 (9 M+2), 294 (22 M^+), 252 (100), 225 (10), 210 (35), 176 (38)
6o	(DMSO-d6): 2.95–3.01 (m, 4H, CH_2) 3.05–3.06 (m, 4H, CH_2) 6.50 (d, 2H, Ar–H, J = 8.0 Hz) 6.70 (d, 2H, Ar–H, J = 8.0 Hz) 6.75 (s, 1H, CH)	3399 (NH, amide); 1648 (CO, amide)	297 (5 M+2), 295 (10 M^+), 252 (100), 210 (60)
6p	($CDCl_3$): 3.25–3.31 (m, 8H, CH_2) 6.25 (s, 1H, CH) 6.60–7.02 (m, 9H, Ar–H)	1660 (CO, amide)	373 (9 M+2), 371 (19 M^+), 294 (28), 252 (100), 238 (50), 223 (76), 211 (28), 161 (47), 119 (40)
6q	(DMSO-d6): 1.24 (t, 3H, CH_3, J = 7.2 Hz) 2.90–3.60 (m, 8H, CH_2) 4.10 (q, 2H, CH_2, J = 7.2 Hz) 6.26 (s, 1H, CH) 7.00–7.40 (m, 4H, Ar–H)	1719 (CO, ester); 1662 (CO, amide)	369 (5 M+2), 367 (14 M^+), 337 (12), 322 (100), 252 (63), 176 (20)
6r	(DMSO-d6): 2.07–2.90 (m, 4H, CH_2) 3.30–3.40 (m, 4H, CH_2) 5.95 (broad, 2H, NH_2) 6.27 (d, 2H, Ar–H, J = 8.1 Hz) 6.48 (d, 2H, Ar–H, J = 8.1 Hz) 6.72 (s, IH, CH)	3370, 3190 (NH_2, amide); 1659 (CO, amide)	340 (11 M+2), 338 (30 M^+), 294 (100), 210 (30), 130 (15)

[a] Spectral data are in accordance with values given in [22].
[b] Spectral data are in accordance with values given in [11].
[c] Spectral data are in accordance with values given in [23].

Table 3
Yields and physicochemical properties of the dithiodipropionamides **4a–s** prepared

Compound	Yield (%)	M.p. (°C)	Formula
4a	83.0	181 [a]	
4b	86.0	206–209 [b]	
4c	79.0	173–174 [c]	
4d	83.0	184–185	$C_{20}H_{18}F_6N_2O_2S_2$
4e	73.0	157–158 [d]	
4f	42.6	165–167	$C_{20}H_{24}N_2O_2S_2$
4g	73.0	183–185	$C_{20}H_{24}N_2O_4S_2$
4h	33.7	174–176	$C_{22}H_{28}N_2O_4S_2$
4i	56.0	177–178	$C_{24}H_{32}N_2O_4S_2$
4j	48.6	161–164	$C_{22}H_{30}N_4O_2S_2$
4k	69.0	166–167	$C_{22}H_{24}N_2O_6S_2$
4l	80.0	170–172 [e]	
4m	90.0	164 [f]	
4n	64.0	183–187	$C_{22}H_{22}N_8O_4S_2$
4o	37.0	164–166	$C_{26}H_{36}N_6O_2S_2$
4p	57.0	168	$C_{38}H_{44}N_6O_2S_2$
4q	75.6	176–179	$C_{38}H_{44}N_6O_2S_2$
4r	49.4	168–172	$C_{28}H_{38}N_8O_4S_2$
4s	53.6	192–196	$C_{20}H_{24}N_2O_2S_4$

[a] Ref. [11] gives 179–184 °C.
[b] Ref. [11] gives 204–210 °C.
[c] Ref. [11] gives 172–174 °C.
[d] Ref. [11] gives 157–160 °C.
[e] Ref. [11] gives 169–171 °C.
[f] Ref. [11] gives 165–166 °C.

It has been reported that 5-chloro-2-alkyl-3(2H)-isothiazolones have higher antibacterial and antifungal activity than the corresponding C-5 unsubstituted analogues [1], and by the theoretical studies on 5-chloro-2-methyl-3(2H)-isothiazolone, the main active component of the commercial biocide kathon, it has been concluded that additional mode of action may be responsible for very high activity of this compound. In contrast to results of these reports, some 5-chloro derivatives of the present study in comparison with the corresponding unsubstituted analogues were less active against *S. aureus* (**6e**), *S. epidermidis* (**6c** and **6d**), *B. subtilis* (**6b–6h, 6j**), *E. coli* (**6b** and **6d**), *S. typhimurium* (**6b** and **6j**) and *P. aeruginosa* (**6b**) or had comparable activities against *S. aureus* (**6h**), *E. coli* (**6c** and **6h**) and *S. typhimurium* (**6c**).

Comparison of the data in the Table 5 show that with exception of the good activity of **6a** against both strains (MIC 0.15–0.6 μg/ml) and high activity of **6d** against *P. aeruginosa*, (MIC 0.1 μg/ml), other compounds with electron withdrawing groups on the phenyl ring (**6b–d**) were more active against Gram-positive and less active against Gram-negative bacteria.

Similar to quinolone antibacterials in which introduction of the piperazine derivatives enhance their activities against Gram-negative bacteria [20], with the exception of the low activity of **6r** against *P. aeruginosa* (MIC 50 μg/ml), other compounds of this study substituted at the 4-position of the phenyl moiety with these derivatives (**6o–r**) had higher activities against Gram-negative in comparison with Gram-positive bacteria. This observation is in agreement with the known tendency of Gram-negative bacteria toward more hydrophilic compounds and inefficacy of lipophilic antibiotics against these organisms.

Findings of this study about the equal activity of compounds with distinctive differences in electronic, size and hydrophobicity such as **6b, 6e, 6m, 6o, 6q** (MIC 0.3 μg/ml) on *S. epidermidis* or **6b, 6j, 6n, 6p** (MIC 2.5 μg/ml) on *B. subtilis* reveal that the activity is not exclusively controlled by a single parameter and each parameter has its own importance. Results of this study show that the nature of substituents on the phenyl ring is determinant for the nature and extent of the activity of the synthesized compounds and might have influences on their inhibitory mechanism of ac-

Table 4
Spectroscopic data of dithiodipropionamides **4a–s**

Compound	IR (cm^{-1})	Mass m/e EI (relative abundance %)
4a	3308 (NH, amide); 1680 (CO, amide)	215 (50), 181 (19), 127 (100)
4b	3354 (NH, amide); 1700 (CO, amide); 1506, 1301 (NO_2)	225 (20), 193 (25), 138 (55)
4c	3308 (NH, amide); 1721 (CO, ester); 1649 (CO, amide)	253 (100), 219 (25), 165 (20)
4d	3329 (NH, amide); 1675 (CO, amide)	248 (30), 215 (35), 188 (12)
4e	3283 (NH, amide); 1659 (CO, amide)	180 (95), 148 (60), 92 (100)
4f	3277 (NH, amide); 1654 (CO, amide)	195 (100), 161 (10)
4g	3270 (NH, amide); 1649 (CO, amide)	211 (40), 177 (40), 123 (100), 107 (85)
4h	3303 (NH, amide); 1649 (CO, amide); 1239 (CO, ether)	224 (45), 191 (25), 136 (58), 107 (100)
4i	3270 (NH, amide); 1653 (CO, amide); 1248 (CO, ether)	238 (65), 205 (30), 163 (21), 151 (62), 108 (100)
4j	3288 (NH, amide); 1649 (CO, amide)	224 (100), 190 (59), 135(100), 119 (38)
4k	3393 (NH, amide); 1716 (CO, ester); 1654 (CO, amide)	238 (43), 205 (20), 196 (65), 163 (78), 109 (100)
4l	3311 (NH, amide); 1690 (CO, amide)	104 (100)
4m	3268 (NH, amide); 1650 (CO, amide)	194 (40), 162 (45), 106 (100)
4n	3303, 3295 (NH, amide); 1706, 1685 (CO, amide)	263 (28), 231 (22), 221 (10), 203 (15), 194 (25), 176 (100), 161 (15)
4o	3530 (NH, amide); 1646 (CO, amide)	264 (83), 232 (100), 218 (23), 204 (10), 176 (14)
4p	3310 (NH, amide); 1653 (CO, amide)	341 (100), 309 (22), 252 (18), 236 (30), 173 (42)
4q	3276 (NH, amide); 1695 (CO, ester); 1661 (CO, amide)	336 (10), 304 (18), 291 (10), 276 (10), 259 (100), 248 (58)
4r	3380, 3340 (NH_2, amide); 3298 (NH, amide); 1659 (CO, amide)	307 (100), 275 (23), 259 (16), 247 (20), 231 (10)
4s	3277 (NH, amide); 1654 (CO, amide)	227 (55), 193 (25), 139 (100)

Table 5
In vitro antibacterial activities of 3(2H)-isothiazolones **5a–j, 5s** and **6a–r** and standard drugs gentamycin, ceftazidime, ceftriaxone, ciprofloxacin (MIC in μg/ml)

Compound	*S. aureus* (ATCC 29737)	*S. epidermidis* (ATCC 12229)	*B. subtilis* (ATCC 12711)	*E. coli* (ATCC 8739)	*S. typhimurium* (ATCC 19430)	*P. aeruginosa* (ATCC 9027)
5a	3.25	1.63	0.8	7.5	7.5	10
5b	3.25	7.5	0.4	30	7.5	50
5c	20	1.25	0.5	50	50	20
5d	20	0.2	0.2	20	20	20
5e	3.25	3.25	0.16	12.5	25	12.5
5f	20	2	0.2	20	20	20
5g	7.5	3.25	0.8	25	12.5	20
5h	10	1	0.5	5	20	2.5
5i	20	2	1	20	20	20
5j	20	2	0.125	20	20	20
5s	20	2	0.125	20	20	20
6a	0.3	0.15	0.3	0.3	0.3	0.6
6b	2.5	0.3	2.5	50	50	100
6c	12.5	2.5	5	50	50	10
6d	5	5	10	25	12.5	0.1
6e	50	0.3	50	0.6	0.3	1.25
6f	5	0.5	1	5	5	0.01
6g	1.25	0.25	1.25	1	1.25	0.01
6h	10	0.5	10	5	10	2
6i	2.5	1	0.25	10	10	10
6j	2.5	0.5	2.5	2.5	25	1.25
6k	5	2.5	10	1.25	10	0.01
6l	1.25	2.5	1.25	1	1	0.1
6m	0.3	0.3	0.6	0.6	12.5	25
6n	50	1.25	2.5	2.5	0.6	2.5
6o	12.5	0.3	12.5	0.3	0.3	0.3
6p	50	50	2.5	2.5	1.25	2.5
6q	50	0.3	50	0.3	0.3	0.3
6r	25	2.5	1.25	0.6	0.6	50
Gentamycin	2.5	0.125	1.25	5	10	10
Ceftazidime	10	0.6	50	0.25	0.5	2.5
Ceftriaxone	5	5	5	0.01	0.01	50
Ciprofloxacin	0.5	1	0.01	0.01	0.01	0.01

tions. Further development of this group of compounds may lead to compounds with better pharmacological profile than the standard drugs.

5. Experimental

Melting points were determined on a Reichert hot plate and are uncorrected. ^{1}HNMR spectra were recorded on a Varian Unity Plus 400 spectrometer (300.866 MHz) using *DMSO-d_6* and *$CDCl_3$* as solvent. Chemical shifts (δ) are reported in ppm relative to *TMS* as internal standard. Mass spectra were obtained on a Finningan TSQ-70 instrument. Infrared spectra were recorded on a Nicolet Magna IR 550 spectrometer. Elemental analyses for C, H and N were performed using a Heracus CHN–O-rapid elemental analyzer and the results are within ±0.4% of the theoretical values.

Amines **3a–h, 3j, 3l, 3m** and **3s** were purchased from the commercial suppliers. 4-propoxy benzeneamine **3i**, 4-aminophenyl acetate **3k**, 4-piperazine-1-yl-phenylamine **3o**, 4 (4-phenyl-piperazine-1-yl)-phenylamine **3p**, 4-(4-aminophenyl)-piperazine-1-carboxylic acid ethyl ester **3q** and 4(4-aminophenyl)-piperazine-1-carboxamide **3r** were prepared by the reported methods, respectively [12–17]. The novel 4-(4-aminophenyl)-2H-1, 2,4-triazole-3(4H)-one **3n** was synthesized in 23% yield following the procedure described for the preparation of the analogous 4-(4-bromophenyl)-2H-1,2,4-triazole-3(4H)-one using 1,4-phenylenediamine as the starting amine instead [18] (m.p. 165–168 °C).

^{1}HNMR ($CDCl_3$, 80 MHz): 5.13 (s, 2H, NH_2) 6.6 (d, 2H, Ar–H, J = 8.8 Hz) 7.18 (d, 2H, Ar–H, J = 8.8 Hz) 8.05 (s, 1H, CH) 11.7 (broad, 1H, NH). Mass *m/z* (relative abundance %): 176 (100), 149 (5), 136 (50), 119 (100), 107 (95). Anal. found: C, 54.56; H, 4.54; N, 31.81; O, 9.07. Calculated for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80; O, 9.08.

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