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Synthesis and characterization of sulfide, sulfoxide and sulfone derivatives of thiopyran: antimicrobial evaluation

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Abstract A series of thiopyran derivatives and their oxidized analogous forms were synthesized and characterized by FT-IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectroscopy techniques. The antibacterial and antifungal activities of these synthesized materials were evaluated against *Staphylococcus aureus* and *Bacillus subtilis*, as Gram-positive bacteria, and *Escherichia coli* and *Pseudomonas aeruginosa*, as Gram-negative bacteria, as well as the fungus *Candida albicans*. The results revealed that thiopyran S,S-dioxides are the most effective against all the bacteria studied in this work. Furthermore, thiopyran S-oxides showed excellent antifungal activity against *Candida albicans*.

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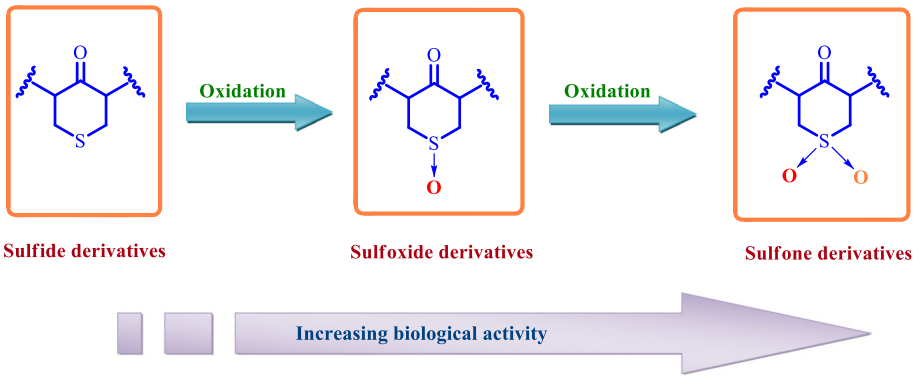
Graphical Abstract



Keywords Antibacterial · Antifungal · Thiopyran S-oxides · Thiopyran S · S-dioxides · Thiopyrans

Introduction

Blocking the growth and multiplication of bacteria is an important action for antimicrobial agents. Many infectious diseases, caused by a variety of pathogens, can be controlled by antimicrobials [1]. Resistance to antimicrobial agents is increasing rapidly, and, therefore, designing new and potential antimicrobial agents with low risk of toxicity is a crucial issue [2]. The compounds possessing heterocyclic moieties have attracted much interest due to their biological and medicinal activities [3, 4]. Thiopyrans, six-membered heterocyclic compounds, have occupied an important place in the realm of natural and synthetic organic chemistry. They are key units in the medicinal chemistry and are widely used in the organic synthesis as versatile building blocks of biological active compounds [5, 6]. Thiopyran derivatives have shown efficient antimicrobial activity in a wide range of pharmaceutical and medicinal chemistry [7–13]. For instance, compounds **A**, **B** and **C** (Fig. 1) have been reported as antibacterial [14], anti-inflammatory [15], and anticancer [16] reagents, respectively. Thiopyran derivatives are also used in the synthesis of various bioactive compounds with potential biological activities, such as serricornin [17], tetrahydrodicranenone B [18], cyclopentanoids [19] and thromoboxanes [20]. Furthermore, it has been reported that oxidation of thiopyran sulfides to S-oxides and S,S-dioxides can significantly increase the biological activities of thiopyrans [15, 21–27]. For example, the compounds **D**, **E** and **F** (Fig. 1) have shown higher biological activities than their sulfide analogous forms.

In the continuation of our research interest in the synthesis of potentially bioactive heterocyclic compounds [28–30], we report here on the synthesis of thiopyran**s** and their derivatives for the evaluation of their antibacterial and

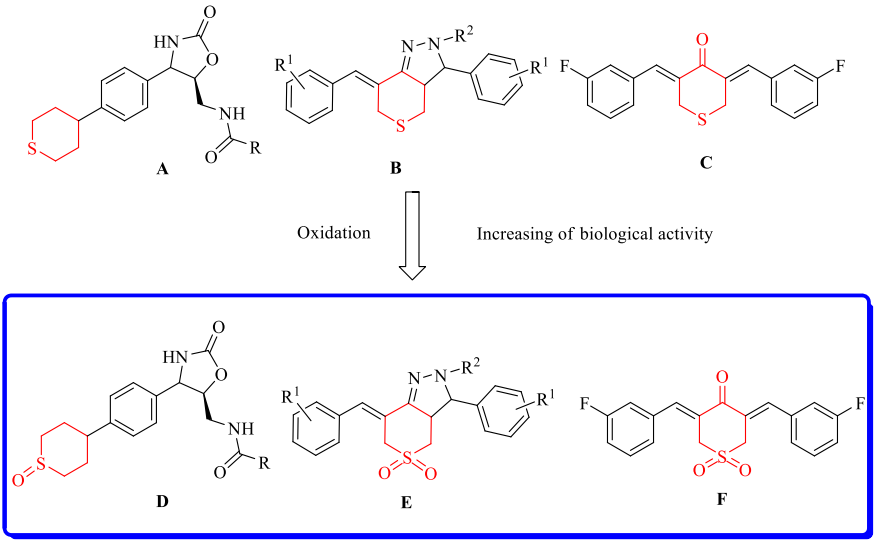


Fig. 1 Some biologically active thiopyran derivatives

antifungal activities. Firstly, thiopyrans **1a**–**5a** and their derivatives of S-oxides **1b**–**5b** and S,S-dioxides **1c**–**5c** were prepared. Then, their antibacterial and antifungal activities against *Staphylococcus aureus* and *Bacillus subtilis*, as Gram-positive bacteria, as well as *Escherichia coli* and *Pseudomonas aeruginosa*, as Gram-negative bacteria, and the fungus *Candida albicans* were evaluated.

Experimental

All solvents, *meta*-chloroperoxybenzoic acid (*m*-CPBA), methyl-chloroformate, lithium diisopropylamide (LDA), triphenylphosphine (PPh_3), dimethyl acetylenedicarboxylate, 3,3′-thiodipropionic acid and other inorganic chemicals including H_2SO_4, Na_2SO_4 and Na_2CO_3, were purchased from Merck and were used without further purification. The compounds **1a–5a** were prepared according to the reported methods [31–33]. Sulfoxides **1b–5b** and sulfone derivatives **1c–5c** were prepared from their corresponding sulfides by *m*-CPBA according to the reported methods [27]. The uncorrected melting points were measured by an Electrothermal 9100 apparatus (UK). The FT-IR spectra were recorded on a FT-IR Bruker Vector 22 spectrometer. Mass spectra were recorded on a Finnigan-Matt 8430 mass spectrometer operating in electron impact mode. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker 400 MHz Ultrashield Advance DRX spectrometer in $CDCl_3$ at 400.1, 100.6 and 169.1 MHz, respectively. Elemental analyses were performed using a Heraeus CHN-O rapid analyzer. The spectral data for the newly synthesized compounds are presented in the supplementary data.

General procedure for the synthesis of thiopyran-S-oxides 1b–5b

A solution of *m*-CPBA (1.0 mmol) in AcOEt (2 mL) was added to the solution of **1a–5a** (1.0 mmol) in dichloromethane (DCM) (10 mL) dropwise at 5 °C and stirred for 30 min at this temperature. The resulting solution was diluted with 20 mL of DCM, washed with 3 × 10 mL of saturated solution of Na_2CO_3, dried over anhydrous Na_2SO_4, and concentrated under vacuum to give S-oxides **1b–5b** in excellent yields as white solids (Table 1).

The spectral data for the newly synthesized sulfoxides **3b–5b** are as follows.

*Dimethyl 4-oxotetrahydro-2H-thiopyran-3,5-dicarboxylate 1-oxide (**3b**, $C_9H_{12}O_6S$)*

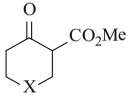
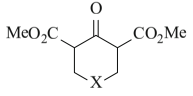
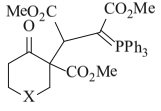
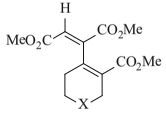
White powder, m.p: 121–122 °C, yield 95%,; IR (KBr) (υ_{max}, cm^{-1}): 3436 (OH), 1733 and 1702 (C=O), 1242 (C^2_{sp}-O), 1115 (C^3_{sp}-O), 1107 (SO); MS, *m/z* (%): 248 (M^+) (3), 232 (20), 216 (8), 200 (26), 173 (100), 155 (5), 141 (43), 113 (100), 86 (41), 55 (100); Anal. Calcd for $C_9H_{12}O_6S$ (248.04): C, 43.54; H, 4.87, Found: C, 43.48; H, 4.86; ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H (for enol tautomer) 2.94 (1H, dd, $^3J_{HH}$ = 14.0, $^2J_{HH}$ = 4.8 Hz, CH_2), 3.01–3.11 (1H, m, CH_2), 3.28–3.50 (2H, m, CH_2), 3.62 (0.67H, dd, $^3J_{HH}$ = 6.0 and 4.8 Hz, CH), 3.77, 3.80, 3.82 and 3.82 (12H, s, 4OCH_3), 4.15 (0.32H, dd, $^3J_{HH}$ = 10.0 and 4.8 Hz, CH), 12.59 (0.67H, s, OH), 12.79 (0.32H, s, OH); δ_H (for keto tautomer) 2.94 (1H, dd, $^3J_{HH}$ = 14.0, $^2J_{HH}$ = 4.8 Hz, CH_2), 3.01–3.11 (1H, m, CH_2), 3.28–3.50 (2H, m, CH_2), 3.62 (0.67H, dd, $^3J_{HH}$ = 6.0 and 4.8 Hz, CH), 3.78 and 3.81 (6H, s, 2OCH_3), 4.15 (0.32H, dd, $^3J_{HH}$ = 10.0 and 4.8 Hz, CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C (for enol tautomer) 23.5, 27.9, 32.6 and 32.7 (4CH_2), 44.2 and 45.2 (2CH), 52.4, 52.5 and 53.2 (4OCH_3), 90.1 and 98.7 (2C_q), 166.7, 168.3, 170.1, and 171.7 (4C=O, ester), 171.8 (2C_q); δ_C (for keto tautomer), 39.8 and 46.9 (2CH_2), 52.1 and 52.7 (2OCH_3), 57.6 and 59.5 (2CH), 167.9 and 170.3 (2C=O, ester), 199.4 (C=O, ketone).

*Dimethyl 2-[3-(methoxycarbonyl)-1-oxido-4-oxotetrahydro-2H-thiopyran-3-yl]-3-(triphenylphosphoranylidene)succinate (**4b**, $C_{31}H_{31}O_8PS$)*

White powder, m.p. 149–150 °C, yield 95%; IR (KBr) (υ_{max}, cm^{-1}): 3059 (C_{sp2}-H), 1723 (C=O), 1628 and 1483 (C=C), 1198 (C^2_{sp}-O), 1122 (C^3_{sp}-O), 1107 (SO); MS, *m/z* (%): 594 (M^+) (2), 405 (8), 347 (3), 294 (32), 277 (100), 236 (11), 201 (19), 183 (61), 152 (18), 77 (20); Anal. Calcd for $C_{31}H_{31}O_7PS$ (594.14): C, 62.62; H, 5.25, Found: C, 62.48; H, 5.23.

Diastereomer I-*Z*-isomer, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.56–2.63 (1H, m, CH_2), 2.98 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.16–3.26 (2H, m, CH_2), 3.72 (3H, s, OCH_3), 3.52–3.54 (1H, m, CH_2), 3.81–3.83 (1H, m, CH_2), 3.97 (1H, d, $^3J_{HP}$ = 16.4 Hz, CH), 4.30–4.35 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 34.1 (CH_2), 38.5 (d, $^1J_{PC}$ = 124.8 Hz, P = C), 42.6 (CH_2), 45.6 (CH_2), 48.9 (d, $^2J_{PC}$ = 14.6 Hz, CH), 48.9, 52.2, and 52.5 (3OCH_3), 64.7 (C_q), 127.5 (d, $^1J_{PC}$ = 91.8 Hz, C_{ipso}), 128.6 (d, $^2J_{PC}$ = 12.0 Hz,

Table 1 The evaluation of antimicrobial activities of compounds **1–5**

Structure	Compounds[a]	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
	**1a**	NE[b]	NE	NE	7.5 ± 0.7	NE
	1b	8.0 ± 1.4	NE	8.5 ± 0.7	7.5 ± 0.7	15.0 ± 1.0
	1c	14.0 ± 1.4	12.5 ± 0.7	10.5 ± 0.7	11.5 ± 0.7	8.3 ± 0.6
	**2a**	7.5 ± 0.7	7.5 ± 0.7	8.0 ± 1.4	8.0 ± 1.4	16.0 ± 1.4
	2b	9.0 ± 1.4	8.0 ± 1.4	9.0 ± 1.4	11.5 ± 0.7	18.3 ± 0.6
	2c	30.5 ± 0.7	22.5 ± 0.7	27.5 ± 0.7	23.0 ± 1.4	NE
	**3a**	NE	NE	7.5 ± 0.7	7.5 ± 0.7	NE
	3b	8.0 ± 1.4	8.0 ± 0.7	9.0 ± 0.7	8.0 ± 1.4	16.7 ± 0.6
	3c	18.5 ± 0.7	15.5 ± 0.7	26.5 ± 0.7	20.5 ± 0.7	NE
	**4a**	NE	NE	NE	NE	NE
	4b	8.5 ± 0.7	9.0 ± 1.4	10.0 ± 0.7	10.0 ± 1.4	12.7 ± 0.6
	4c	9.0 ± 1.4	11.5 ± 0.7	20.0 ± 1.4	20.5 ± 0.7	7.3 ± 0.6
	**5a**	9.0 ± 1.4	NE	9.0 ± 1.4	11.5 ± 0.7	NE
	5b	10.5 ± 0.7	12.0 ± 1.4	9.5 ± 0.7	11.5 ± 0.7	NE
	5c	10.5 ± 0.7	14.5 ± 0.7	15.0 ± 1.4	17.5 ± 0.7	8.3 ± 0.6
Standard antibiotics	Gentamicin (10 μg/disc)	19.6 ± 1.1	15.6 ± 0.5	20.3 ± 1.5	26.0 ± 1.7	–
	Chloramphenicol (30 μg/disc)	20.7 ± 1.5	NE	21.7 ± 0.6	22.3 ± 1.2	–
	Nystatin (100 Units/disc)	–	–	–	–	11.3 ± 0.6

[a] X = S (**1a–5a**), X = SO (**1b–5b**), X = SO_2(**1c–5c**), [b] no effect (NE = 6 mm)

C_{ortho}), 132.0 (d, $^{4}J_{PC}$ = 2.5 Hz, C_{para}), 133.8 (d, $^{3}J_{PC}$ = 10.0 Hz, C_{meta}), 169.1 (d, $^{2}J_{PC}$ = 7.1 Hz, C=O, ester), 170.3 (C=O, ester), 174.0 (d, $^{3}J_{PC}$ = 5.0 Hz, C=O, ester), 199.2 (C=O, ketone); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ_P 24.5.

Diastereomer I-*E*-isomer, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.56–2.63 (1H, m, CH_2), 2.95 (3H, s, OCH_3), 3.16–3.26 (2H, m, CH_2), 3.30 (3H, s, OCH_3), 3.52–3.54 (1H, m, CH_2), 3.74 (3H, s, OCH_3), 3.81–3.83 (1H, m, CH_2), 3.93 (1H, d, $^{3}J_{HP}$ = 14.0 Hz, CH), 4.30–4.35 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 34.9 (CH_2), 38.5 (d, $^{1}J_{PC}$ = 124.8 Hz, P = C), 44.3 (CH_2), 45.6 (CH_2), 48.9 (d, $^{2}J_{PC}$ = 14.6 Hz, CH), 50.8, 52.3 and 53.1 ($3OCH_3$), 65.8 (C_q), 127.5 (d, $^{1}J_{PC}$ = 91.8 Hz, C_{ipso}), 128.6 (d, $^{2}J_{PC}$ = 12.0 Hz, C_{ortho}), 132.0 (d, $^{4}J_{PC}$ = 2.5 Hz, C_{para}), 133.8 (d, $^{3}J_{PC}$ = 10.0 Hz, C_{meta}), 170.4 (C=O, ester), 174.5 (d, $^{3}J_{PC}$ = 4.0 Hz, C=O, ester), 175.1 (d, $^{2}J_{PC}$ = 7.0 Hz, C=O, ester), 201.1 (C=O, ketone); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ_P 24.70.

Diastereomer II-*Z*-isomer, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.56–2.63 (1H, m, CH_2), 3.00 (3H, s, OCH_3), 3.16–3.26 (2H, m, CH_2), 3.31 (3H, s, OCH_3), 3.52–3.54 (1H, m, CH_2), 3.67 (3H, s, OCH_3), 3.77–3.81 (1H, m, CH_2), 3.97 (1H, d, $^{3}J_{HP}$ = 16.4 Hz, CH), 4.36–4.39 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons);^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 33.6 (CH_2), 37.6 (d, $^{1}J_{PC}$ = 124.3 Hz, P = C), 42.6 (CH_2), 45.1 (CH_2), 48.5 (d, $^{2}J_{PC}$ = 14.5 Hz, CH), 49.1, 50.8 and 54.6 ($3OCH_3$), 64.8 (C_q), 127.5 (d, $^{1}J_{PC}$ = 91.8 Hz, C_{ipso}), 128.5 (d, $^{2}J_{PC}$ = 12.2 Hz, C_{ortho}), 131.9 (d, $^{4}J_{PC}$ = 2.8 Hz, C_{para}), 133.7 (d, $^{3}J_{PC}$ = 10.5 Hz, C_{meta}), 169.1 (d, $^{2}J_{PC}$ = 7.1 Hz, C=O, ester), 169.7 (C=O, ester), 170.4 (d, $^{3}J_{PC}$ = 5.0 Hz, C=O, ester), 199.3 (C=O, ketone); δ_P 24.3.

Diastereomer II-*E*-isomer, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.56–2.63 (1H, m, CH_2), 3.00 (3H, s, OCH_3), 3.16–3.26 (2H, m, CH_2), 3.21 (3H, s, OCH_3), 3.52–3.54 (1H, m, CH_2), 3.74 (3H, s, OCH_3), 3.77–3.81 (1H, m, CH_2), 3.93 (1H, d, $^{3}J_{HP}$ = 14.0 Hz, CH), 4.36–4.39 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons);^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 34.2 (CH_2), 37.6 (d, $^{1}J_{PC}$ = 124.3 Hz, P = C), 44.4 (CH_2), 45.1 (CH_2), 48.5 (d, $^{2}J_{PC}$ = 14.5 Hz, CH), 50.7, 51.9, and 54.8 ($3OCH_3$), 66.8 (C_q), 127.5 (d, $^{1}J_{PC}$ = 91.8 Hz, C_{ipso}), 128.5 (d, $^{2}J_{PC}$ = 12.2 Hz, C_{ortho}), 131.9 (d, $^{4}J_{PC}$ = 2.8 Hz, C_{para}), 133.7 (d, $^{3}J_{PC}$ = 10.5 Hz, C_{meta}), 169.8 (C=O, ester), 170.1 (d, $^{2}J_{PC}$ = 7.2 Hz, C=O, ester), 170.9 (d, $^{3}J_{PC}$ = 4.0 Hz, C=O, ester), 203.1 (C=O, ketone); δ_P 24.55.

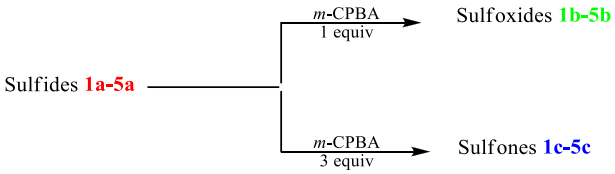
*Dimethyl (2E)-2-[5-(methoxycarbonyl)-1-oxido-3,6-dihydro-2H-thiopyran-4-yl]but-2-enedioate (**5b**, $C_{13}H_{16}O_7S$)*

Yellow powder, m.p. 98–99 °C, yield 90%; IR (KBr) (υ_{max}, cm^{-1}): 1723 and 1650 (C=O), 1610 (C=C), 1251 (C^{2}_{sp}-O), 1186 (SO), 1115 (C^{3}_{sp}-O); MS, *m/z* (%): 316 (M^{+})(3), 277 (100), 257 (2), 199 (31), 183 (27), 152 (18), 77 (30), 51 (18); Anal. Calcd for $C_{13}H_{16}O_7S$ (332.06): C, 49.36; H, 5.10, Found: C, 49.42; H, 5.12; ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.52–2.64 (1H, m, CH_2), 2.97–3.02 (1H, m, CH_2), 3.11–3.14 (2H, m, CH_2), 3.69 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.75 (2H, m, CH_2), 3.82 (3H, s, OCH_3), 6.77 (1H, s, olefinic proton);^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 24.8, 43.2 and 46.3 ($3CH_2$), 52.0, 52.2 and 53.1 ($3OCH_3$), 124.3

(quaternary olefinic carbon), 124.7 (olefinic carbon, CH), 145.4 and 147.3 (quaternary olefinic carbon), 164.7, 164.9 and 165.4 (3C=O, ester).

General procedure for the synthesis of sulfones 1c–5c

A solution of *m*-CPBA (3.0 mmol) in AcOEt (5 mL) was added to the solution of **1a–5a** (1.0 mmol) in dichloromethane (DCM) (20 mL) dropwise at 5 °C and stirred for 30 min at this temperature. The resulting solution was diluted with 30 mL of DCM, washed with 3 × 10 mL of saturated solution of Na_2CO_3, dried over anhydrous Na_2SO_4, and concentrated under vacuum to give S,S-dioxides **1c–5c** in excellent yields as white solids (Table 2).



The spectral data for the newly synthesized sulfoxides **3c–5c** are as follows.

*Dimethyl 4-oxotetrahydro-2H-thiopyran-3,5-dicarboxylate 1,1-dioxide (**3c**, $C_9H_{12}O_7S$)*

White powder, m.p. 115 °C, yield 95%; IR (KBr) (υ_{max}, cm^{-1}): 3481 (OH), 1738 and 1710 (C=O), 1379 and 1142 (SO_2), 1226 (C^2_{sp}-O), 1122 (C^3_{sp}-O); MS, *m/z* (%): 265 (3), 233 (5), 200 (3), 168 (16), 140 (9), 114 (21), 87 (56), 55 (100); Anal. Calcd for $C_9H_{12}O_7S$ (248.04): C, 40.91; H, 4.58, Found: C, 40.82; H, 4.59; ^{1}H NMR (400.1 MHz, $CDCl_3$): (for enol tautomer): δ_H 3.22–3.28 (1H, m, CH_2), 3.46–3.52 (1H, m, CH_2), 3.60–3.65 (1H, m, CH), 3.84 and 3.85 (6H, s, $2OCH_3$), 4.03–4.08 (1H, m, CH), 12.59 (1H, s, OH): (for keto tautomer): δ_H 3.22–3.28 (1H, m, CH_2), 3.46–3.52 (1H, m, CH_2), 3.60–3.65 (1H, m, CH), 3.85 (6H, s, OCH_3), 4.03–4.08 (1H, m, CH), 12.59 (1H, s, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C (for enol tautomer) 47.6 (CH), 48.0 and 49.8($2CH_2$), 52.8 and 53.4 ($2OCH_3$), 93.2 (C_q), 165.7 and 167.9 (2C=O, ester), 170.0 (C_q), (for keto tautomer), 46.7 (2CH), 49.0 ($2CH_2$), 53.3 ($2OCH_3$), 170.7 (C=O, ester), 202.8 (C=O, ketone).

Table 2 Preparation of the sulfoxides **1b–5b** by *m*-CPBA

Compounds	*m*-CPBA	Products	m.p	References
1a	1 equiv	**1b**	110	[39]
2a	1 equiv	**2b**	84	[40]
3a	1 equiv	**3b**	121–122	This work
4a	1 equiv	**4b**	149–150	This work
5a	1 equiv	**5b**	98–99	This work

*Dimethyl 2-[3-(methoxycarbonyl)-1,1-dioxido-4-oxotetrahydro-2H-thiopyran-3-yl]-3-(triphenylphosphoranylidene)succinate (**4c**, $C_{31}H_{31}O_9PS$)*

White powder, m.p. 160–161 °C, yield 95%; IR (KBr) (υ_{max}, cm^{-1}): 3058 (C_{sp2}-H), 1720 (C=O), 1625 and 1481 (C=C), 1435 and 1109 (SO_2), 1244 (C^2_{sp}-O), 1061 (C^3_{sp}-O); MS, *m/z* (%): 609 (M^+-1) (1), 337 (12), 316 (4), 294 (47), 277 (100), 236 (4), 201 (16), 183 (66), 152 (18), 113 (18), 77 (17); Anal. Calcd for $C_{31}H_{31}O_7PS$ (610.14): C, 60.98; H, 5.12, Found: C, 60.78; H, 5.10.

4c-Z-isomer, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.55–2.59 (1H, m, CH_2), 2.99 (3H, s, OCH_3), 3.10–3.26 (2H, m, CH_2), 3.31 (3H, s, OCH_3), 3.36–3.45 (1H, m, CH_2), 3.74 (3H, s, OCH_3), 3.81–3.82 (1H, m, CH_2), 3.96 (1H, d, $^3J_{HP}$ = 15.6 Hz, CH), 4.30–4.39 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 33.9 (CH_2), 38.5 (d, $^1J_{PC}$ = 123.7 Hz, P = C), 42.5 (CH_2), 44.3 (CH_2), 48.9 (OCH_3), 49.0 (d, $^2J_{PC}$ = 14.8 Hz, CH), 52.2 and 53.1 ($2OCH_3$), 65.8 (C_q), 127.5 (d, $^1J_{PC}$ = 94.8 Hz, C_{ipso}), 128.5 (d, $^2J_{PC}$ = 12.0 Hz, C_{ortho}), 132.0 (d, $^4J_{PC}$ = 2.2 Hz, C_{para}), 133.9 (m, C_{meta}), 170.2 (d, $^2J_{PC}$ = 12.8 Hz, C=O, ester), 170.9 (C=O, ester), 170.0 (d, $^3J_{PC}$ = 7.0 Hz, C=O, ester), 201.1 (C=O, ketone); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ_P 24.5.

***4c-E**-isomer*, ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.55–2.59 (1H, m, CH_2), 2.94 (3H, s, OCH_3), 3.01 (3H, s, OCH_3), 3.10–3.26 (2H, m, CH_2), 3.29 (3H, s, OCH_3), 3.36–3.45 (1H, m, CH_2), 3.81–3.82 (1H, m, CH_2), 3.92 (1H, d, $^3J_{HP}$ = 16.0 Hz, CH), 4.30–4.39 (1H, m, CH_2), 7.51–7.74 (15H, m, aromatic protons);^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 33.6 (CH_2), 37.5 (d, $^1J_{PC}$ = 116.0 Hz, P = C), 44.4 and 44.6 ($2CH_2$), 48.9 (OCH_3), 50.6 (d, $^2J_{PC}$ = 15.0 Hz, CH), 50.8 and 52.3 ($2OCH_3$), 64.8 (C_q), 127.5 (d, $^1J_{PC}$ = 94.8 Hz, C_{ipso}), 128.6 (d, $^2J_{PC}$ = 11.9 Hz, C_{ortho}), 132.1 (d, $^4J_{PC}$ = 2.2 Hz, C_{para}), 133.9 (m, C_{meta}), 169.0 (d, $^2J_{PC}$ = 12.8 Hz, C=O, ester), 171.8 (C=O, ester), 174.0 (d, $^3J_{PC}$ = 5.0 Hz, C=O, ester), 199.2 (C=O, ketone); δ_P 24.55.

*Dimethyl (2E)-2-[5-(methoxycarbonyl)-1,1-dioxido-3,6-dihydro-2H-thiopyran-4-yl]but-2-enedioate (**5c**, $C_{13}H_{16}O_8S$)*

Yellow powder, m.p. 83–85 °C, yield 95%; IR (KBr) (υ_{max}, cm^{-1}): 1724 and 1648 (C=O), 1617 (C=C), 1449 and 1118 (SO_2), 1252 (C^2_{sp}-O), 1050 (C^3_{sp}-O); MS, *m/z* (%): 331 (M^+-H) (2), 301 (6), 273 (53), 240 (67), 225 (10), 209 (84), 193 (23), 176 (15), 149 (34), 121 (18), 84 (100), 59 (37); Anal. Calcd for $C_{13}H_{16}O_8S$ (332.06): C, 46.98; H, 4.85, Found: C, 46.85; H, 4.86; ^{1}H NMR (400.1 MHz, $CDCl_3$): δ_H 2.89–3.06 (2H, m, CH_2), 3.15–3.37 (2H, m, CH_2), 3.68 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.94 and 4.03 (2H, AB, $^2J_{HH}$ = 17.6 Hz, CH_2), 6.73 (1H, s, olefinic proton);^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 32.3, 46.7 and 50.1 ($3CH_2$), 52.3, 52.5 and 53.2 ($3OCH_3$), 120.1 (quaternary olefinic carbon), 124.7 (olefinic carbon, CH), 145.8 and 147.7 (quaternary olefinic carbon), 164.3, 164.4 and 165.1 (3C=O, ester).

General procedure for the evaluation of antimicrobial activity

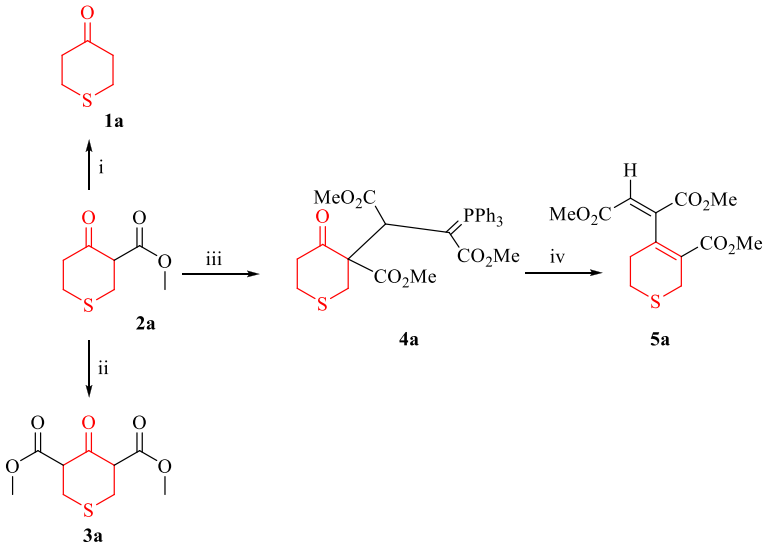
The most widespread method for the assessment of antimicrobial activities is the Kirby–Bauer disc diffusion technique [42]. In vitro antimicrobial activities of the compounds **1–5** were assayed using the disc diffusion method by determining the inhibition zones. The microorganisms, used in this study, are as follows: *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* PTCC 1074, *Staphylococcus aureus* ATCC 35923, *Bacillus subtilis* PTCC 1023 and *Candida albicans* ATCC 10231. The late exponential phases of the bacteria were standardized with a final cell density of 10^8 cfu/mL. Muller–Hinton agar (Merck) were prepared and inoculated by the standardized cultures of the studied microorganisms, spread as uniformly as possible throughout the entire media. Sterile paper discs (diameter of 6 mm; Padtan, Iran) were impregnated with 20 mL of the sample solution (20 mg/mL in DMSO), and then placed on the upper layer of the seeded agar plate and incubated at 37 °C for 24 h. The antimicrobial activities of the samples were compared with the known commercial antibiotics of gentamicin (10 µg/disc), chloramphenicol (30 µg/disc) and nystatin (100 Units/disc). After the incubation period, the antibacterial and antifungal activities were estimated by calculating the mean diameter (mm) of the inhibition halo, where the tested microorganism did not grow, and the results were reported as mean ± SD after three repeats.

Results and discussion

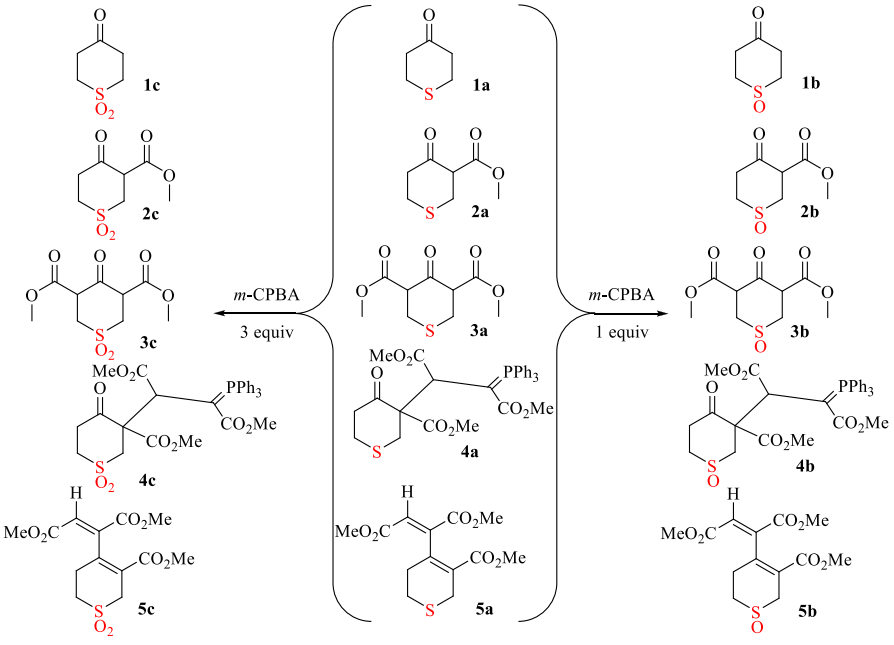
Chemistry

Syntheses of the compounds **1a–5a** are depicted in Scheme 1. Compound **2a** was prepared by the Dieckmann cyclization reaction of dimethyl 3,3′-thiodipropanoate in the mixture of NaOMe/THF according to the reported procedure [31]. Treatment of compound **2a** with H_2SO_4 (10%) under refluxing conditions afforded **1a** in high yield [31]. The reaction of compound **2a** with methyl-chloroformate in the presence of LDA at −78 °C led to the formation of compound **3a** in good yield [32]. The three-component reaction of thiopyran sulfide **2a** with dimethyl acetylenedicarboxylate and triphenylphosphine resulted in phosphorous ylide **4a**, which converted to compound **5a** in excellent yield when refluxed in toluene by the intramolecular Wittig reaction [33].

It is known that the oxidation of sulfides to higher oxidation states, i.e., S-oxides and S,S-dioxides, usually improves their biological activities [21–24] Therefore, all the synthesized sulfides in this work were oxidized to their corresponding sulfoxides and sulfones. The oxidation of sulfides **1a–5a** to sulfoxides **1b–5b** and sulfones **1c–5c** is presented in Scheme 2. As is clear from Scheme 2, when one equivalent of *m*-CPBA is used for the oxidation of sulfides **1a–5a**, the corresponding sulfoxides **1b–5b** are obtained as sole products in excellent yields without the formation of any other oxidation products. Similarly, the sulfones **1c–5c** are synthesized using three equivalents of *m*-CPBA. The structures of all the synthesized compounds were characterized by FT-IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectroscopy techniques. The



Scheme 1 Preparation of compounds **1a–5a**. Reagents and conditions: (*i*) 10% H_2SO_4, reflux for 1 h (*ii*) LDA, methyl-chloroformate, THF, −78 °C (*iii*) triphenylphosphine and dimethylacetylenedicarboxylate, DCM, r.t., 20 min (*iv*) toluene, reflux for 2 h



Scheme 2 Preparation of S-oxide and S,S-dioxide derivatives of sulfides **1a–5a**

mass spectra of these compounds exhibited the molecular ion peaks at the appropriate regions. Other fragmentations involved the loss of the ester or alkoxy groups from the molecular ions. The FT-IR spectra of sulfoxides **1b–5b** and sulfones **1c–5c** displayed strong absorption bands at 1720–1650 cm^{-1} for the carbonyl groups. The S=O stretching vibrational peak for the sulfoxides appeared at 1100 cm^{-1} and the symmetric and asymmetric stretching peaks of SO_2 group of sulfones displayed at 1110 and 1400 cm^{-1}. In the ^1H NMR ($CDC1_3$), the methylene (CH_2X) protons appear as multiplets at approximate δ values of 2.7–2.9 and 3.3–3.7 (X = S), 2.9–3.0 and 3.8–4.3 (X = SO), 2.9–3.1 and 3.9–4.4 (X = SO_2) ppm, which could be due to the X-substituent effect. These methylene groups (CH_2X) displayed δ values at 20–30 ppm (X = S), 25–35 (X = SO), and 30–40 (X = SO_2) ppm in the ^{13}C NMR spectra in agreement with the proposed structures.

Biological evaluation

Antibacterial activity

The contamination and microbial infection caused by the microorganisms, the increasing number of microbial strains resistant to many antibiotics, and faster growth of some microorganisms than antibiotics are important concerns for the human beings. Therefore, design and synthesis of new reagents for the treatment of microbial infections are challenging tasks [34, 35]. One type of antimicrobial agents are thiopyran derivatives offering potential biological activities [36, 37]. In order to investigate the biological activities of all the synthesized thiopyran heterocycles, their antibacterial and antifungal activities against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and a fungus (*Candida albicans*) were evaluated in vitro using the Kirby–Bauer disk diffusion method. The results are summarized in Table 3 and compared with the gentamicin, chloramphenicol and nystatin as standard drugs. In general, all the samples showed a range of modest to highest in vitro antibacterial activity against all the tested microorganisms. However, compounds **1a** against the studied Gram-negative bacteria and *S. aureus*, **1b** against *P. aeruginosa*, **3a** against the Gram-negative bacteria, **4a** against all tested four bacteria, and **5a** against *P. aeruginosa* did not show antibacterial activity. Compound **1a** without any substituent at the alpha position, at the C_3 and C_5 positions of the six-membered heterocyclic ring, did not exhibit in vitro antibacterial

Table 3 Preparation of the sulfones **1c–5c** by *m*-CPBA

Compounds	*m*-CPBA	Products	m.p	References
1a	3 equiv	**1c**	174–175	[41]
2a	3 equiv	**2c**	115	[41]
3a	3 equiv	**3c**	115	This work
4a	3 equiv	**4c**	160–161	This work
5a	3 equiv	**5c**	83–85	This work

activity except against *B. subtilis*. In compound **1a**, the introduction of a methyl carboxylate group (CO_2Me) at the C_3 position of the thiopyran-4-one moiety resulted in **2a**, slightly improving the antibacterial activity against all four bacteria. When another methyl carboxylate group was substituted at the C_5 position of the thiopyran-4-one moiety, this resulted in **3a**, and the activity against all bacteria did not significantly change in comparison to the antibacterial activity of compound **2a**. Replacement of hydrogen at the C_3 position in **2a** by a dimethyl 2-(triphenylphosphoranylidene) succinate group resulted in **4a**, which suppressed the antimicrobial activity against all the tested microorganisms. Removing the carbonyl and phosphine oxide from **4a** through the intramolecular Wittig reaction led to the formation of **5a** which significantly enhanced the antibacterial activities against all the bacteria except *P. aeruginosa*. In general, the comparison of antibacterial activities of the compounds **1a–5a** against the four tested bacteria, as shown in Fig. 2(I), revealed that compound **5a** had the highest activity against the two studied Gram-positive bacteria and *E. coli*, a Gram-negative bacterium, whereas compound **2a** showed the highest effect against *P. aeruginosa*, a Gram-positive bacterium.

The antibacterial activities of the S-oxide derivatives **1b–5b** exhibited the same trend as the thiopyrans **1a–5a** with just a slight improvement. Comparison of the antibacterial activities of the sulfoxide derivatives **1b–5b** against all the tested bacteria, as shown in Fig. 2(II), showed more growth inhibitory effects for

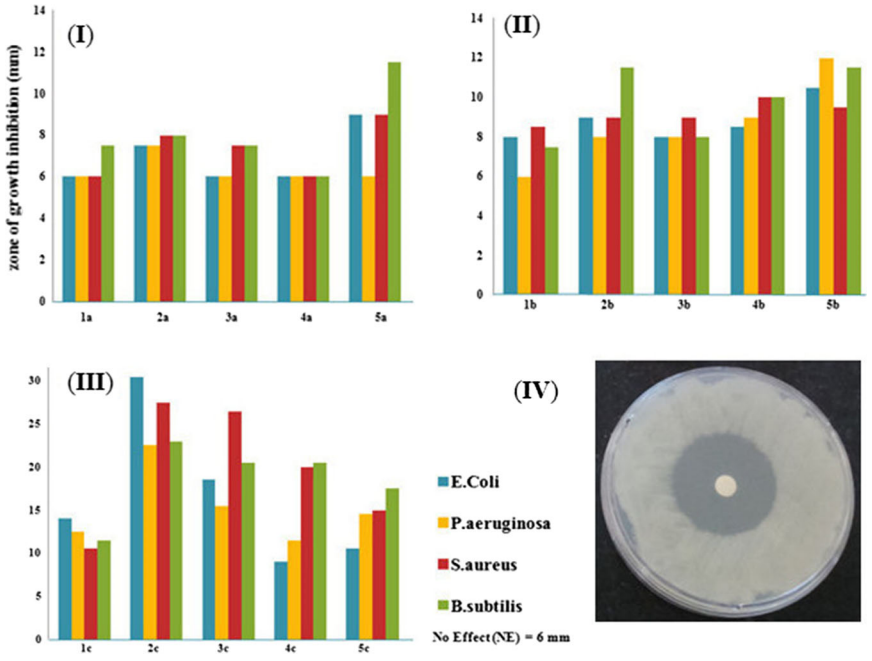


Fig. 2 Comparison of the antibacterial activities of sulfide derivatives **1a–5a** (**I**), S-oxide derivatives **1b–5b** (**II**), S,S-dioxide derivatives **1c–5c** (**III**) and the growth inhibitory effect of **2c** on *E. coli* using the disk diffusion test (**IV**)

compound **5b** against the two Gram-negative bacteria, for compound **4b** against *P. aeruginosa*, and for compounds **2b** and **5b** against *B. subtilis*. The antibacterial activities of the S,S-dioxide derivatives **1c–5c** released the maximum activity against all the tested bacteria. In compound **1c**, the introduction of an ester group (CO_2Me) at the C_3 position of the thiopyran-4-one resulted in compound **2c**, which impressively increased the antibacterial activity. Furthermore, by the introduction of a second ester group at the C_5 position of thiopyran-4-one, this resulted in compound **3c**, and the antibacterial activity decreased against all the studied bacteria in comparison to that observed for **2c**. Here, the antibacterial activities of these materials were in the order: **2c** > **3c** > **1c**. Similar trends were observed from the comparisons between **2a** with **3a**, as well as **2b** with **3b**. In compound **1c**, the replacement of two hydrogens in the alpha-position at the C_3 position of thiopyran-4-one by a dimethyl 2-(triphenylphosphoranylidene) succinate and an ester group gave **4c**, which improved the activity against the Gram-positive bacteria and decreased the antibacterial activity against the Gram-negative bacteria. Interestingly, comparing the antibacterial activities of **4c** with **5c** demonstrated an increase in the activity against the Gram-negative bacteria as well as a decrease against the Gram-positive bacteria. Figure 2(III) shows the antibacterial activities of the S,S-dioxide derivatives **1c–5c**. Accordingly, compound **2c** had the highest growth inhibitory effect against all the examined microorganisms. The growth inhibitory effect of **2c** on *E. coli* using the disk diffusion test is shown in Fig. 2(IV).

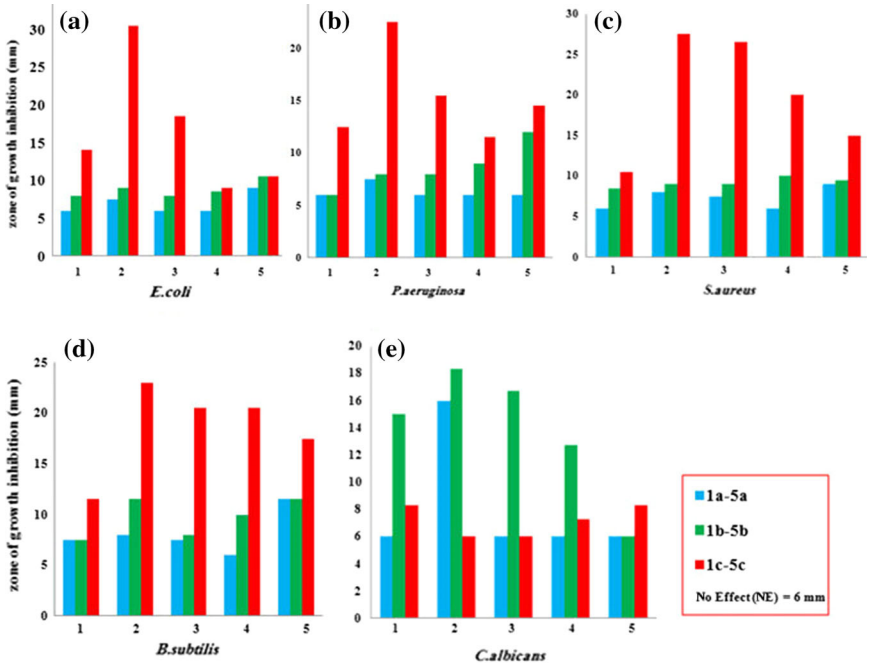


Fig. 3 Antimicrobial activity against Gram-negative bacteria *E. coli* (**a**), and *P. aeruginosa* (**b**), Gram-positive bacteria *S. aureus* (**c**), and *B. subtilis* (**d**) and the fungus *C. albicans* (**e**)

The obtained results revealed that the thiopyran S,S-dioxides with sulfur in its highest oxidation state had potential antimicrobial activities in comparison with their corresponding derivatives with sulfur in its lower oxidation states. This trend of antibacterial activity enhancement has also been observed in other sulfur-containing heterocycles [15, 21–25]. The comparison of the antibacterial activities of thiopyran sulfides, thiopyran S-oxides and thiopyran S,S-dioxides against the four bacteria examined in this study are depicted in Fig. 3a–d. Among the studied compounds, compound **2c** showed excellent antibacterial activity against all four tested bacteria which is comparable with the activity of standard antibiotics (gentamicin and chloramphenicol). It seems that the presence of the thiopyran-4-one-S,S-dioxide moiety and one ester functional group in the backbone of compounds **1c–5c** acted as antibacterial agents. It is suggested that the binding ability to cell wall components increases via hydrogen bonding in the presence of a sulfone functional group and, as a result, the lysis of the bacterial cells and the leakage of the cytoplasmic materials increase, which leads to the cell death of bacteria [15, 21–27].

Antifungal activity

The in vitro antifungal activities of all the compounds **1–5** were investigated against *Candida albicans* (Table 3). Nystatin was used as a standard antibiotic whose growth inhibitory effect value was reported in Table 3. Among thiopyrans **1–4**, thiopyran-4-one-S-oxides **1b–4b** showed higher growth inhibitory activity against *C. albicans* than against Nystatin. Compounds **1c**, **2a** and **4c** exhibited moderate antifungal activities while compounds **1a**, **2c**, **3a**, **3c** and **4a** did not show any antifungal activities. Among thiopyrans **5a–c**, only **5c** showed moderate antifungal activity against *C. albicans* (Fig. 3e). It is noteworthy that sulfoxide derivatives seem to be primarily active against *C. albicans*. Generally, different structural parameters and mechanisms of action are responsible for achieving potency against a range of microorganisms [38].

Conclusions

Some derivatives of thiopyran heterocycles can be used as antimicrobial agents against important human pathogenic microorganisms, such as *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans*, which play a significant role in deadly infectious diseases. Based on our results, a structure–activity correlation can be provided. The structure containing one ester group (CO_2Me) at the C3 position of the thiopyran-4-one-S,S-dioxide plays an important role in eliciting the biological response. Also, the oxidation of sulfur to higher states in the six-membered S-heterocycles, i.e., sulfide to sulfone, improves the antibacterial activity, whereas the improvement for the antifungal activity is seen with the thiopyran S-oxides.

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