Mol Divers
DOI 10.1007/s11030-017-9737-8

ORIGINAL ARTICLE

Synthesis, antioxidant and antimicrobial activities of novel thiopyrano[2,3-*d*]thiazoles based on aroylacrylic acids

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Received: 29 September 2016 / Accepted: 4 April 2017

Abstract Here it is described the synthesis, antioxidant and antimicrobial activity determination of novel *rel*-(5*R*, 6*S*, 7*R*)-6-benzoyl-7-phenyl-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*]thiazole-5-carboxylic acids. The target compounds were obtained in good yields from 5-arylidene-4-thioxo-2-thiazolidinones and β-aroylacrylic acids via regio- and diastereoselective *hetero*-Diels–Alder reaction. The stereochemistry of the cycloaddition was confirmed by NMR spectra. The antioxidant and antimicrobial activity screening identified 7 compounds (**3c**, **3e**, **3f**, **3g**, **3k**, **3l**, **3p**) with a high level of free radical scavenging (43–77% DPPH assay), and compounds with significant influence on *Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* (MIC 3.13–6.25 μg/mL), but slight effect on *Escherichia coli*.

Electronic supplementary material The online version of this article (doi:10.1007/s11030-017-9737-8) contains supplementary material, which is available to authorized users.

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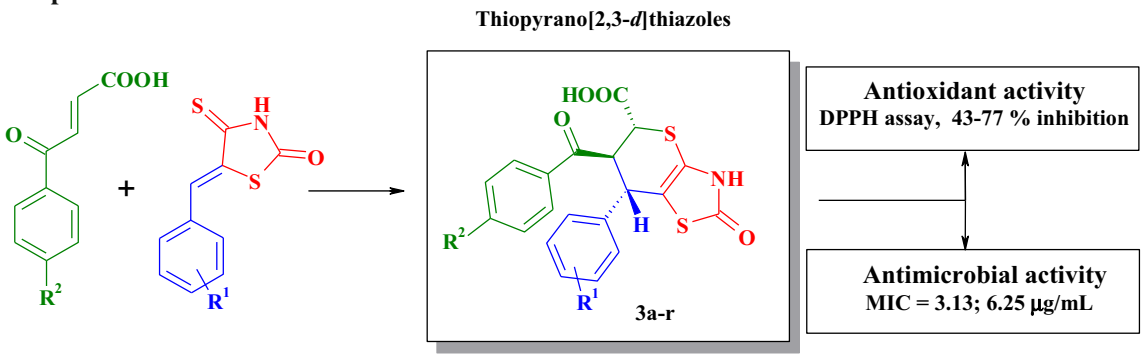
Published online: 19 April 2017

Graphical abstract



Keywords *Hetero*-Diels–Alder reaction · Thiopyrano[2,3-*d*]thiazoles · Antioxidant activity · Antimicrobial activity

Introduction

Thiazolidinones, as privileged structures, are of special interest in modern medicinal chemistry due to a wide range of biological activities as well as synthetic features [1,2]. Anticancer [3,4], antimicrobial [5,6], antiinflammatory [7,8], and antioxidant agents [9,10] have been identified among 4-thiazolidinone derivatives. A majority of biologically active 4-thiazolidinones belongs to a 5-ene-derivative subtype. Thus, the statement about the impact of the substituent at the C5 position (namely 5-ene) of the 4-thiazolidinone core on biological activity realization [3,11,12] was proposed. The conjugation of a C5 exocyclic double bond to the C4 carbonyl group of the thiazolidinone core makes 5-ene-4-thiazolidinones to be possible Michael acceptors. Based on such Michael acceptor functionality, 5-ene-4-thiazolidinones are assigned as frequent hitters or pan assay interference compounds (PAINS) that are often discarded in modern drug discovery [13]. The annulation of 5-ene-4-thiazolidinones into the thiopyrano[2,3-*d*]thiazole system removes the unwanted Michael acceptor functionality and keeps pharmacological profiles similar to 5-ene-4-thiazolidinones. Thus, thiopyrano[2,3-*d*]thiazoles could be considered as cyclic isosteric mimetics of 5-ene-4-thiazolidinones [14–19]. Thiopyrano[2,3-*d*]thiazoles are also a source of new anticancer [14–17], antimicrobial [18], antiinflammatory [19], and antitrypanosomal [20] agents.

The main synthetic protocol for thiopyrano[2,3-*d*]thiazoles is based on the *hetero*-Diels–Alder reaction between mentioned above 5-ene-4-thioxothiazolidines (as heterodienes) and various dienophiles (e.g., acrylonitrile, acrylic acid and its analogs, maleic and fumaric acids derivatives, arylidene pyruvic and cinnamic acids derivatives, norbornene derivatives). Such dienophiles can be considered as a tool for the diversification of thiopyrano[2,3-*d*]thiazoles [14–20]. Moreover, this *hetero*-Diels–Alder reaction is one of the stages of different domino and tandem processes in the synthesis of complex heterocycles [20–22].

This work is an extension of our ongoing efforts toward the synthesis of new substituted thiopyrano[2,3-*d*]thiazoles via a *hetero*-Diels–Alder reaction using β-aroylacrylic acids as dienophiles (Fig. 1). The reaction of β-aroylacrylic acids, which are polyfunctional molecules [23–26], with nucleophiles has been investigated [12,27,28]; however, their utilization as dienophiles in the *hetero*-Diels–Alder reaction has not been studied.

Results and discussion

Chemistry

The synthesis of target thiopyrano[2,3-*d*]thiazoles was performed according to the protocol outlined in Scheme 1. 5-Arylidene-4-thioxo-2-thiazolidinones (5-arylideneisorhodanines) **1a–g** were obtained from reacting 4-thioxo-2-thiazolidinone and the appropriate aldehydes under Knoevenagel condensation conditions: using glacial acetic acid in the presence of sodium acetate (method A) or using ethanol in the presence of ethylenediamine diacetate (EDDA) (method B) [16,21]. β-Aroylacrylic acids (**2a–e**) were obtained from the Friedel–Crafts acylation of arenes and maleic anhydride [29]. The thiopyrano[2,3-*d*]thiazoles synthesis was carried out in boiling acetic acid in the presence of catalytic amount of hydroquinone as a side polymerization inhibitor and desired *rel-(*5*R*, 6*S*, 7*R*)-6-benzoyl-7-phenyl-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*]thiazole-5-carboxylic acids **3a–r** were obtained with good yields.

The structure and stereochemistry of the synthesized compounds were confirmed by spectral data. Thus, the thiopyran protons in the ^{1}H NMR spectra appear as a triplet and two doublets at ~3.73–5.87 ppm with appropriate coupling constants between 9.9 and 10.8 Hz that prove an axial–axial interaction of the 5-H, 6-H and 6-H, 7-H proton pairs. The signals in the low field ~11.40–11.60 ppm were assigned to the amide protons. The ^{13}C

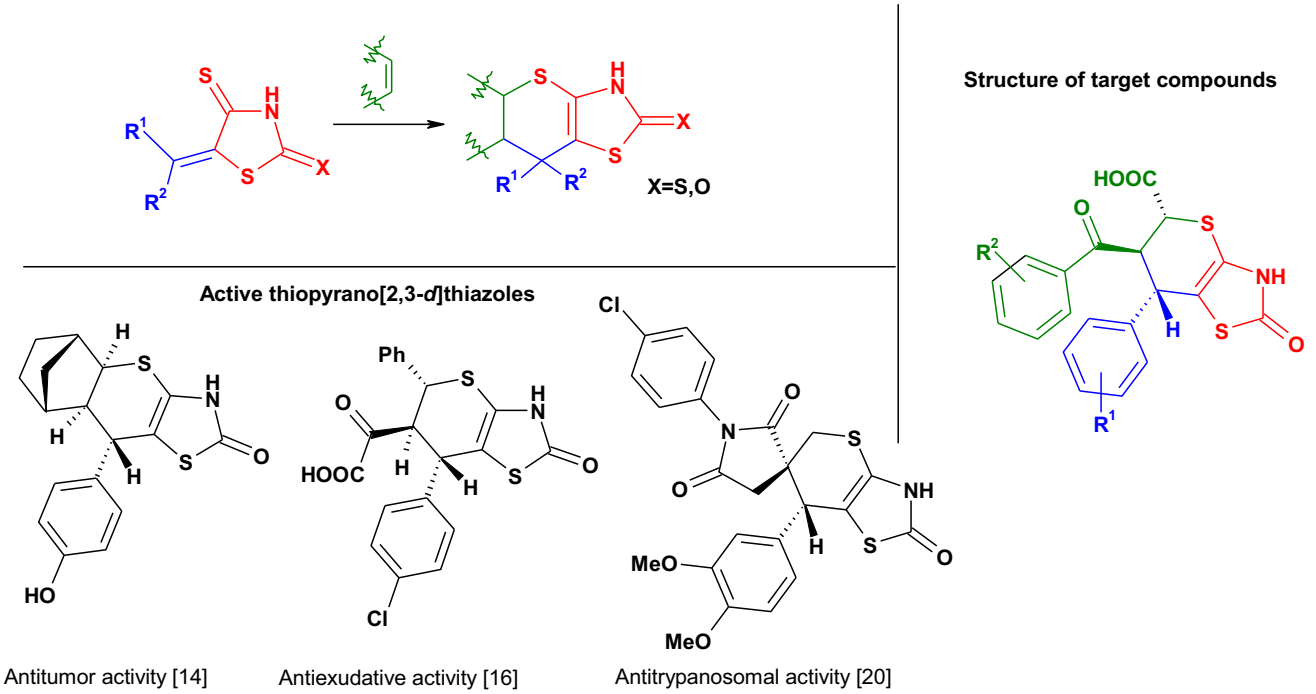


Fig. 1 Background for target compounds synthesis

NMR spectra of compounds show signals at 170–200 ppm which are attributed to carbonyl groups. Similar signals were observed for thiopyrano[2,3-*d*]thiazoles obtained from pyruvic, fumaric, and cinnamic acids [16,17,20,21]. For **3a**, the ^{1}H NMR signals of H-7 and H-6 appeared as a doublet at 4.10 ppm and a triplet at 4.42 ppm, respectively, and correlated with C-17 (200.0 ppm) and C-11 (133.7 ppm), C-12 (128.1 ppm), C-2 (114.7 ppm) and C-6 (107.4 ppm) in HMBC (Fig. 2).

In vitro antioxidant assay

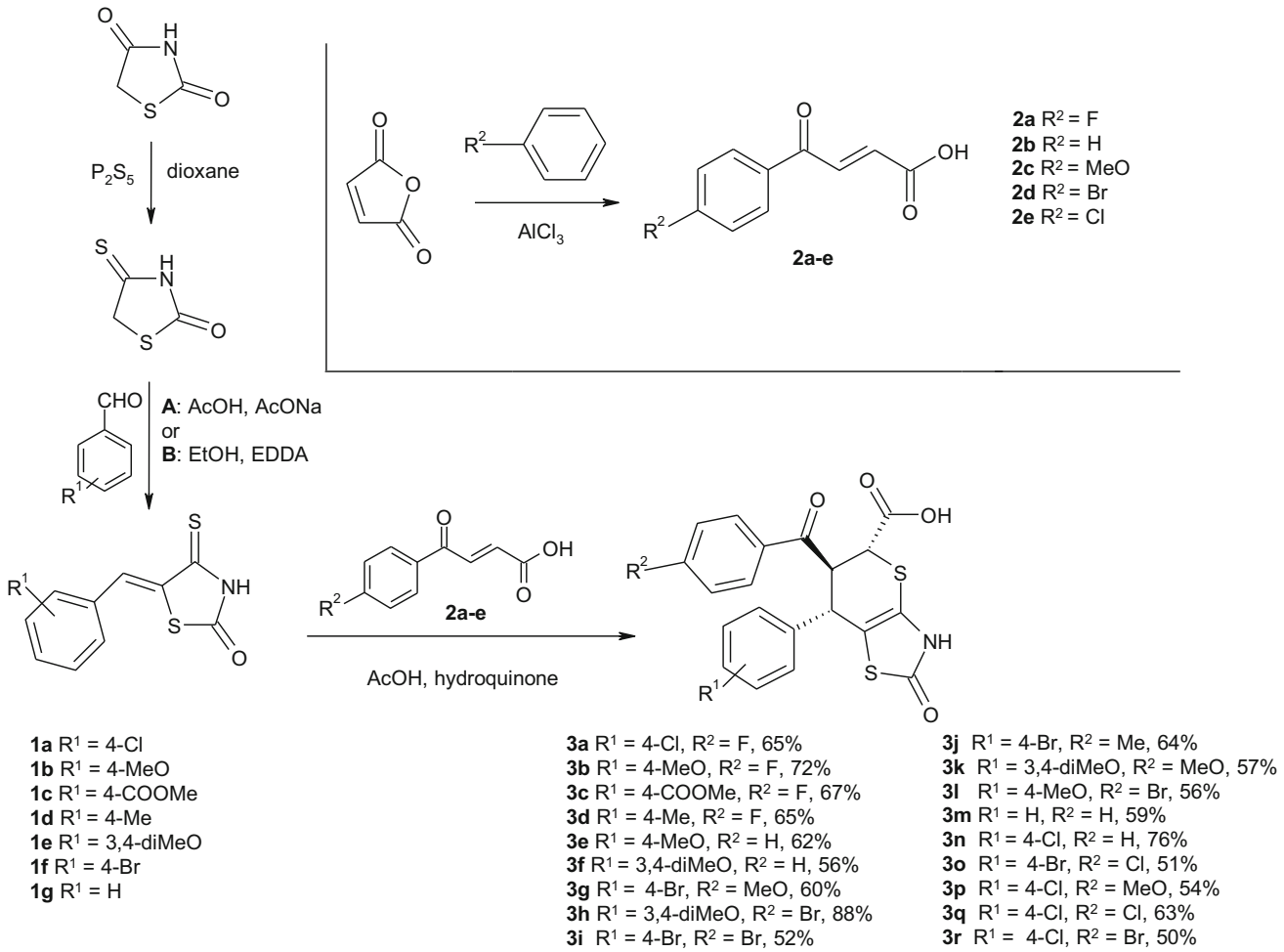
The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay is a well-known method for determination of the antioxidant activity of various compounds. The radical scavenging activity of the tested compounds (final concentration 12.5 μM) ranged from good to moderate compared to L-ascorbic acid (Table 1). Compounds **3c**, **3e–h**, **3k**, **3l** and **3p** stabilize DPPH in the range of 43–77% except of **3a**, **b**, **3d** and **3o**. Our SAR analysis suggests that the presence of substituents (especially methoxy group) in phenyl and benzoyl fragments (position 6 and 7 of thiopyrano[2,3-*d*]thiazole core) is favorable for antioxidant potency. The antioxidant activity of the compounds is probably related to their electron or hydrogen radical releasing ability to DPPH so that they become stable diamagnetic molecules. The antioxidant activity of the compounds can be explained by E_{HOMO} and E_{LUMO}. Compounds that possess higher E_{HOMO} and E_{LUMO} can be more effective agents for stabilizing DPPH radicals [30].

Antimicrobial activity

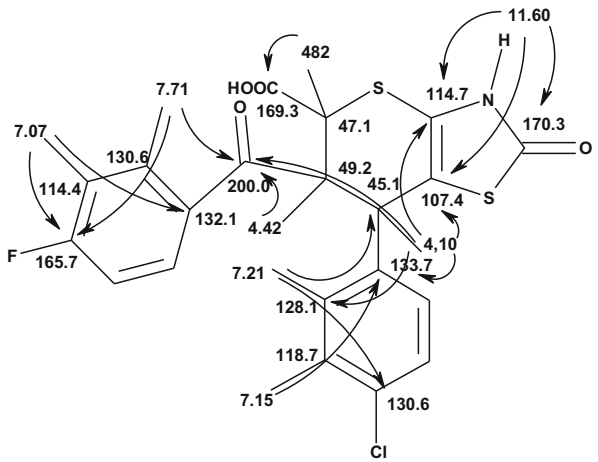
The synthesized compounds were screened for their in vitro antibacterial and antifungal activities using the agar diffusion method. Screening was performed against Gram(+)Ve and Gram(−)Ve bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The obtained data reveal that some of the tested compounds possess good activity toward tested microbial strains at a dose of 20 μg per well (Table 2).

Compound **3l** shows the highest activity against *S. aureus* and *B. subtilis* strains compared to streptomycin (standard). Compounds **3i** and **3l**, **3o–r** show good antibacterial activity against *S. aureus* methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains. Compounds **3h** and **3j** display moderate activity against MRSA. All the test compounds exhibit slight inhibitory activity against *E. coli*. Antifungal activity of the synthesized compounds was performed against *Candida albicans*. Compounds **3h**, **3b**, **3n–q** show better antifungal activities than Amphotericin-B. The minimum inhibitory concentrations for the most active compounds **3b**, **3h–j**, **3l** and **3n–r** against the same microorganisms were calculated using the microdilution susceptibility method [31] (Table 3).

Our SAR study revealed that compounds with electron-withdrawing groups (Cl > Br > F > OCH_3) exhibit



Scheme 1 Synthesis of *rel*-(5*R*, 6*S*, 7*R*)-6-benzoyl-7-phenyl-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*]thiazole-5-carboxylic acids



^{1}H NMR signal, δ, ppm	The cross peaks in ^{13}C NMR	
	HMQC	HMBC
11.60	-	114.7; 107.4; 170.3
7.71	130.6	165.7; 130.6; 200.0; 114.4
7.21	128.1	45.1; 131.6; 128.1
7.15	118.7	133.7; 118.7
7.07	114.4	165.7; 132.1; 114.4;
4.82	47.1	169.3; 49.2; 45.1
4.42	49.2	200.0; 169.3; 133.7; 128.1; 114.7; 107.4; 47.1; 45.1
4.10	45.1	200.0; 133.7; 128.1; 114.7; 107.4; 49.2; 47.1

Fig. 2 Heteronuclear ^{1}H-^{13}C correlations for compound **3a**

higher antimicrobial activity. The data suggest that Cl is a favorable substituent in the phenyl ring at position 7 of the thiopyrano[2,3-*d*]thiazole core. The similar trend was observed for antiviral thiopyrano[2,3-*d*]thiazole-6-carbaldehydes [32].

Table 1 Antioxidant activity of thiopyrano[2,3-*d*]thiazoles

Compound	Absorbance of a sample, A_s	% Inhibition
3a	0.710 ± 0.013	9.00
3b	0.726 ± 0.015	6.88
3c	0.399 ± 0.009	48.81
3d	0.715 ± 0.015	8.39
3e	0.304 ± 0.005	61.00
3f	0.349 ± 0.007	55.29
3g	0.305 ± 0.007	60.96
3h	0.440 ± 0.009	43.57
3i	0.597 ± 0.011	23.49
3j	0.543 ± 0.009	30.40
3k	0.174 ± 0.004	77.71
3l	0.389 ± 0.009	50.07
3m	0.733 ± 0.014	6.00
3n	0.699 ± 0.013	10.36
3o	0.775 ± 0.016	0.61
3p	0.230 ± 0.005	70.49
3q	0.646 ± 0.012	17.18
3r	0.585 ± 0.011	25.02
Ascorbic acid	0.533 ± 0.012	31.70

Data are given as mean ± SD

Conclusions

The new *rel-(5R, 6S, 7R)*-6-benzoyl-7-phenyl-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*]thiazole-5-carboxylic acids were synthesized via a regio- and stereoselective *hetero*-Diels–Alder reaction from appropriate β-aroylacrylic acids and 5-arylideneisorhodanines. The antioxidant activity DDPH assay identified thiopyrano[2,3-*d*]thiazole derivatives with radical scavenging activity in the range of 43–77%. An antimicrobial screening led to the identification of active compounds against *Staphylococcus aureus, Bacillus subtils*, and *Candida albicans* (MIC 3.13–6.25 μg/mL). The results obtained in this work could be used as the basis to explore more thiopyrano[2,3-*d*]thiazole analogs with better antioxidant and antimicrobial properties.

Experimental

Chemistry

Materials and methods

All materials were purchased from commercial sources and used as received. Melting points were measured in open cap-

Table 2 Antimicrobial activity of thiopyrano[2,3-*d*]thiazole-5-carboxylic acids

Compound	Zone of growth inhibition (mm)					
	S. aureus	*S. aureus*	*E. coli*	*B. subtils*	*C. albicans*	
	MSSA	MRSA			Fungistatic	Fungicidal
3a	5.29 ± 0.35	6.19 ± 0.31	5.26 ± 0.28	5.83 ± 0.53	5.27 ± 0.14	4.29 ± 0.09
3b	5.52 ± 0.48	6.81 ± 0.19	5.14 ± 0.28	4.73 ± 0.3	10.38 ± 0.58	7.46 ± 0.22
3c	5.11 ± 0.37	6.42 ± 0.48	4.99 ± 0.48	5.37 ± 0.16	7.58 ± 0.89	6.79 ± 0.63
3d	4.42 ± 0.41	–	4.22 ± 0.41	–	9.04 ± 0.69	5.76 ± 0.33
3f	4.45 ± 0.30	6.35 ± 0.15	4.90 ± 0.27	–	5.08 ± 0.14	4.73 ± 0.21
3g	4.12 ± 0.30	5.60 ± 0.29	4.32 ± 0.59	5.47 ± 0.36	7.88 ± 0.59	5.99 ± 0.20
3h	5.70 ± 0.21	10.20 ± 0.82	4.93 ± 0.08	–	13.21 ± 0.46	10.89 ± 0.22
3i	10.24 ± 1.15	11.42 ± 1.31	4.59 ± 0.47	5.24 ± 0.73	6.73 ± 0.57	5.25 ± 0.38
3j	6.78 ± 0.36	10.02 ± 0.42	4.90 ± 0.14	–	6.91 ± 0.52	5.49 ± 0.42
3k	4.03 ± 0.26	–	4.92 ± 0.21	–	5.93 ± 0.57	4.61 ± 0.28
3l	7.78 ± 0.47	18.15 ± 1.11	5.69 ± 0.36	10.55 ± 0.15	7.71 ± 0.38	5.46 ± 0.61
3n	4.74 ± 0.27	5.93 ± 0.14	4.23 ± 0.36	5.13 ± 0.21	8.94 ± 0.53	7.02 ± 0.36
3o	7.48 ± 0.34	13.25 ± 0.99	5.96 ± 1.01	6.83 ± 0.98	11.92 ± 0.24	10.45 ± 0.37
3q	10.16 ± 0.13	9.67 ± 0.35	5.16 ± 0.36	–	9.76 ± 0.52	8.25 ± 0.67
3r	11.56 ± 0.73	9.50 ± 0.58	5.31 ± 0.34	5.34 ± 0.27	7.46 ± 0.54	5.63 ± 0.65
Streptomycin	13.00 ± 0.42	–	10.00 ± 0.36	10.00 ± 0.38	–	–
Amphotericin–B	–	–	–	–	9.00 ± 0.65	–

Data are given as mean ± SD

Table 3 Minimum inhibitory concentration of tested compounds, μg/mL

Microorganism	MIC (μg/mL)								
	3b	**3h**	**3i**	**3j**	**3l**	**3n**	**3o**	**3q**	**3r**
S. aureus/MSSA	>50	>50	25	>50	50	>50	50	25	25
S. aureus/MRSA	>50	25	12.5	25	6.25	>50	12,5	25	25
E. coli	>50	>50	>50	>50	>50	>50	>50	>50	>50
C. albicans	50	3.13	>50	>50	>50	>50	12.5	25	>50

illary tubes on a BÜCHI B-545 melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were performed using a PerkinElmer 2400 CHN analyzer and were within 0.4% of theoretical values. The ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Gemini 400 MHz in DMSO-d_6 using tetramethylsilane as an internal standard (chemical shift values are reported in ppm units, coupling constants (J) are in Hz). Abbreviations are as follows: s—singlet; d—doublet; t—triplet; m—multiplet; br—broad. Chemical shifts are reported in ppm units with use of δ scale. Mass spectra were obtained using electrospray ionization (ESI) techniques on an Agilent 1100 Series LCMS. The purity of all obtained compounds was checked by TLC (silica gel 60 F254 coated plates (Merck), eluent Benzene : EtOAc 2:1).

*General procedure for 5-arylidene-4-thioxo-2-thiazolidinones **1a–g** synthesis*

The starting 4-thioxo-2-thiazolidinone was obtained according to the literature [33]. 5-Arylidene-4-thioxo-2-thiazolidinones (**1a–g**) were prepared under Knoevenagel reaction conditions:

Method A A mixture of 4-thioxo-2-thiazolidinone (10 mmol), an appropriate aldehyde (10 mmol) and sodium acetate (10 mmol) in 10 mL of glacial acetic acid was stirred for 20 min using a water bath (100 °C). The resulting solid was collected by filtration, washed with water, dried and used without further purification.

Method B A mixture of 4-thioxo-2-thiazolidinone (10 mmol), an appropriate aldehyde (10 mmol) and catalytic amount of EDDA in the ethanol (10 mL) was heated under reflux for 10 min. The resulting solid was collected by filtration, washed with ethanol and diethyl ether, dried and used without further purification.

*General procedure for hetero-Diels–Alder products **3a–r** synthesis*

A mixture of an appropriate 5-arylidene-4-thioxo-2-thiazolidinone **1**(10 mmol), β-aroylacrylic acid **2**(11 mmol) and a catalytic amount of hydroquinone (2–3 mg) in glacial acetic acid (10 mL) was heated under reflux for 1 h and then left overnight at room temperature. The precipitate was filtered, washed with methanol (5–10 mL), and the product recrystallized from acetic acid (10–15 mL).

rel-(5R,6S,7R)-7-(4-Chlorophenyl)-6-(4-fluorobenzoyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3a**). Yield 65%, mp 228–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.10 (d, 1H, J = 10.2 Hz, 7-H), 4.42 (t, 1H, J = 10.2 Hz, 6-H), 4.82 (d, 1H, J = 10.2 Hz, 5-H), 7.07 (d, 2H, J = 8.4 Hz, arom.), 7.15 (d, 2H, J = 8.4 Hz, arom.), 7.21 (d, 2H, J = 8.4 Hz, arom.), 7.71 (dd, 2H, J = 4.1, 3.2 Hz, arom.), 11.60 (s, 1H, NH), 13.41 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1, 47.1, 49.2, 107.4, 114.7, 118.7 (d, J_{CF} = 3.0 Hz), 128.1, 130.6, 131.6 (d, J_{CF} = 30.0 Hz, CH, arom.), 132.1, 133.7, 137.9, 165.7 (d, J_{CF} = 200.0 Hz, C, arom.), 169.3 (C=O), 170.3 (C=O), 200.0 (C=O). ESI-MS *m/z* 450/452 $(M+H)^{+}$. Anal. Calcd for $C_{20}H_{13}ClFNO_4S_2$: C, 53.39; H, 2.91; N, 3.11. Found: C, 53.37; H, 2.93; N, 3.10.

rel-(5R,6S,7R)-6-(4-Fluorobenzoyl)-7-(4-methoxyphenyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3b**). Yield 72%, mp 215–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.55 (s, 3H, OCH_3), 3.95 (d, 2H, J = 10.2 Hz, 7-H), 4.36 (t, 1H, J = 10.2 Hz, 6-H), 4.79 (d, 1H, J = 10.2 Hz, 5-H), 6.58 (d, 2H, J = 8.4 Hz, arom.), 6.99 (d, 2H, J = 8.4 Hz, arom.), 7.04 (d, 2H, J = 8.4 Hz, arom.), 7.65 (d, 2H, J = 8.4 Hz, arom.), 11.52 (s, 1H, NH), 13.55 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1, 47.2, 49.4, 54.9, 108.6, 114.8, 118.1 (d, J_{CF} = 3.0 Hz), 129.8, 130.9, 131.1 (d, J_{CF} = 30.0 Hz), 133.8, 133.9, 158.4, 165.5 (d, J_{CF} = 200.0 Hz), 169.4 (C=O), 170.5 (C=O), 200.5 (C=O). ESI-MS *m/z* 446 $(M+H)^{+}$. Anal. Calcd for $C_{21}H_{16}FNO_5S_2$: C, 56.62; H, 3.62; N, 3.14. Found: C, 56.63; H, 3.64; N, 3.12.

rel-(5R,6S,7R)-6-(4-Fluorobenzoyl)-7-(4-methoxycarbonylphenyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3c**). Yield 67%, mp 188–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 4.14 (d, 1H, J = 10.2 Hz, 7-H), 4.48 (t, 1H, J = 10.2 Hz, 6-H), 4.82 (d, 1H, J = 10.2 Hz, 5-H), 6.88 (d, 2H, J = 8.7 Hz, arom.), 7.06 (d, 2H, J = 8.7 Hz, arom.), 7.79 (d, 2H, J = 8.1 Hz, arom.), 8.16 (dd, 2H, J = 4.2, 8,7 Hz, arom.), 11.68 (s, 1H, NH), 13.80 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.6, 47.5, 49.0, 52.1, 104.7, 107.1, 116.3, 118.9 (d, J_{CF} = 3.0 Hz), 128.7, 129.2,

131.7 (d, J_{CF} = 30.0 Hz), 132.3, 133.6, 144.5, 165.7 (d, J_{CF} = 120.0 Hz), 169.8 (C=O), 170.6 (C=O), 199.8 (C=O). ESI-MS *m/z* 474 $(M+H)^+$. Anal. Calcd for $C_{22}H_{16}FNO_6S_2$: C, 55.81; H, 3.41; N, 2.96. Found: C, 55.82; H, 3.42; N, 2.98.

rel-(5R,6S,7R)-6-(4-Fluorobenzoyl)-2-oxo-7-(4-methylphenyl)-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3d**). Yield 65%, mp 230–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.09 (s, 3H, CH_3), 4.00 (d, 1H, J = 10.2 Hz, 7-H), 4.38 (t, 1H, J = 10.2 Hz, 6-H), 4.81 (d, 1H, J = 10.2 Hz, 5-H), 6.87 (d, 2H, J = 7.8 Hz, arom.), 7.02 (d, 2H, J = 7.8 Hz, arom.), 7.07 (d, 2H, J = 8.1 Hz, arom.), 7.68 (d, 2H, J = 8.1 Hz, arom.), 11.52 (s, 1H, NH), 13.52 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.5, 45.5, 47.3, 49.5, 108.4, 114.8, 118.2 (d, J_{CF} = 3.0 Hz), 128.5, 131.0, 133.8, 133.9 (d, J_{CF} = 30.0 Hz, CH, arom.), 135.8, 136.7, 165.6 (d, J_{CF} = 200.0 Hz), 169.4 (C=O), 170.5 (C=O), 200.3 (C=O). ESI-MS *m/z* 430 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}FNO_4S_2$: C, 58.73; H, 3.75; N, 3.26. Found: C, 58.72; H, 3.75; N, 3.24.

rel-(5R,6S,7R)-6-Benzoyl-7-(4-methoxyphenyl)-2-oxo-(4-methylphenyl)-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d] thiazole-5-carboxylic acid (**3e**). Yield 62%, mp 206–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.81 (t, 1H, J = 10.8 Hz, 6-H), 4.14 (d, 1H, J = 10.8 Hz, 7-H), 5.87 (d, 1H, J = 10.8 Hz, 5-H), 6.90 (d, 2H, J = 9.3 Hz, arom.), 7.15 (d, 2H, J = 9.3 Hz, arom.), 7.59 (t, 1H, J = 7.5 Hz, arom.), 7.71 (t, 2H, J = 7.5 Hz, arom.), 8.12 (d, 2H, J = 7.5 Hz, arom.), 11.51 (s,1H, NH), 13.52 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.6, 47.2, 51.2, 54.9, 109.0, 113.8, 115.1, 117.4, 128.9, 129.5, 132.8, 134.5, 136.2, 158.6, 170.4 (C=O), 172.7 (C=O), 194.4 (C=O). ESI-MS *m/z* 428 $(M+H)^+$. Anal. Calcd for $C_{21}H_{17}NO_5S_2$: C, 59.00; H, 4.01; N, 3.28. Found: C, 58.99; H, 4.03; N, 3.29.

rel-(5R,6S,7R)-6-Benzoyl-7-(3,4-dimethoxyphenyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3f**). Yield 56%, mp 188–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (t, 1H, J = 9, 9 Hz, 6-H), 3.75 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.10 (d, 1H, J = 9.9 Hz, 7-H), 5.84 (d, 1H, J = 9.9 Hz, 5-H,), 6.71 (d, 1H, J = 9.9 Hz, arom.), 6.78 (s, 1H, arom.), 6.84 (d, 1H, J = 8.7 Hz, arom.), 7.00 (t, 1H, J = 7.8 Hz, arom.), 7.73 (t, 2H, J = 7.8 Hz, arom.), 8.07 (d, 2H, J = 7.8 Hz, arom.), 11.42 (s, 1H, NH), 13.58 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.5, 43.9, 47.2, 50.9, 55.5, 108.7, 111.6, 117.5, 120.4, 128.1, 128.5, 128.9, 133.2, 134.2, 134.4, 147.9, 148.9, 170.5 (C=O), 170.8 (C=O), 194.7 (C=O). ESI-MS *m/z* 458 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}NO_6S_2$: C, 57.75; H, 4.19; N, 3.06. Found: C, 57.73; H, 4.18; N, 3.04.

rel-(5R,6S,7R)-7-(4-Bromophenyl)-6-(4-methoxybenzoyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3g**). Yield 60%, mp 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 4.16 (d, 1H, J = 10.2 Hz, 7-H), 5.48 (t, 1H, J = 10.2 Hz, 6-H), 5.79 (d, 1H, J = 10.2 Hz, 5-H), 6.94 (d, 2H, J = 8.7 Hz, arom.), 7.09 (d, 2H, J = 8.7 Hz, arom.), 7.21 (d, 2H, J = 8.4 Hz, arom.), 7.33 (d, 2H, J = 8.4 Hz, arom.), 11.41 (s, 1H, NH), 13.35 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.8, 46.7, 50.2, 55.6, 107.7, 113.8, 118.3, 120.4, 127.1, 130.7, 131.5, 139.6, 142.1, 163.1, 170.7 (C=O), 172.6 (C=O), 192.6 (C=O). ESI-MS *m/z* 506/508 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}BrNO_5S_2$: C, 49.81; H, 3.18; N, 2.77. Found: C, 49.82; H, 3.16; N, 2.75.

rel-(5R,6S,7R)-6-(4-Bromobenzoyl)-7-(3,4-dimethoxyphenyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3h**). Yield 88%, mp 112–114 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.76 (t, 1H, J = 10.2 Hz, 6-H), 3.94 (d, 1H, J = 10.2 Hz, 7-H), 4.80 (d, 1H, J =10.2 Hz, 5-H), 6.55 (d, 1H, J =8.1 Hz, arom.), 6.75 (s, 1H, arom.), 6.98 (d, 1H, J =8.1 Hz, arom.), 7.59 (d, 2H, J=8.4 Hz, arom.), 7.97 (d, 2H, J =8.4 Hz, arom.), 11.50 (s, 1H, NH), 13.40 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.5, 47.0, 48.8, 50.6, 55.1, 111.6, 118.0, 120.3, 125.1, 128. 8, 130.0, 131.5, 132.3, 133.5, 134.3, 136.0, 148.2, 169.5 (C=O), 170.6 (C=O), 201.3 (C=O). ESI-MS *m/z* 536/538 $(M+H)^+$. Anal. Calcd for $C_{22}H_{18}BrNO_6S_2$: C, 49.26; H, 3.38; N, 2.61. Found: C, 49.27; H, 3.35; N, 2.60.

rel-(5R,6S,7R)-6-(4-Bromobenzoyl)-7-(4-bromophenyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3i**). Yield 52%, mp 174–176 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.06 (d, 1H, J = 10.2 Hz, 7-H), 4.36 (t, 1H, J = 10.2 Hz, 6-H), 4.85 (d, 1H, J = 10.2 Hz, 5-H), 7.09 (d, 2H, J = 8.4 Hz, arom.), 7.26 (d, 2H, J = 8.4 Hz, arom.), 7.48 (d, 2H, J = 8.7 Hz, arom.), 8.01 (d, 2H, J = 8.7 Hz, arom.), 11.59 (s, 1H, NH), 13.39 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.0, 47.0, 49.2, 107.3, 118.8, 126.8, 130.0, 130.9, 131.1, 131.5, 132.0, 135.9, 138.3, 169.3 (C=O), 170.3 (C=O), 200.7 (C=O). ESI-MS *m/z* 554/556/558 $(M+H)^+$. Anal. Calcd for $C_{20}H_{13}Br_2NO_4S_2$: C, 43.26; H, 2.36; N, 2.52. Found: C, 43.24; H, 2.35; N, 2.50.

rel-(5R,6S,7R)-7-(4-Bromophenyl)-6-(4-methylbenzoyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3j**). Yield 64%, mp 162–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.51 (s, 3H, CH_3), 4.08 (d, 1H, J = 10.5 Hz, 7-H), 4.37 (t, 1H, J = 10.5 Hz, 6-H,), 4.75 (d, 1H, J = 10.5 Hz, 5-H), 7.10 (d, 2H, J = 8.4 Hz, arom.), 7.26 (d, 2H, J = 8.4 Hz, arom.), 7.52 (d, 2H, J = 8.1 Hz, arom.), 7.99 (d, 2H, J = 8.1 Hz, arom.), 11.57 (s, 1H, NH), 13.58 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.0, 45.0, 47.4, 49.1, 107.5, 118.7, 120.7, 128.2, 128.5, 130.8, 131.0, 134.4, 138.6, 143.1, 169.2 (C=O), 170.3 (C=O), 200.4 (C=O). ESI-MS *m/z* 490/492 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}BrNO_4S_2$: C, 51.43; H, 3.29; N, 2.86. Found: C, 51.42; H, 3.27; N, 2.85.

rel-(5R,6S,7R)-7-(3,4-Dimethoxyphenyl)-6-(4-methoxylbenzoyl)-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thia-

zole-5-carboxylic acid (**3k**). Yield 57%, mp 184–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.81 (t, 1H, J = 10.5 Hz, 6-H), 3.87 (s, 3H, OCH_3), 4.08 (d, 1H, J = 10.5 Hz, 7-H), 5.77 (d, 1H, J=10.5 Hz, 5-H), 6.72 (d, 1H, J =8.1 Hz, arom.), 6.77 (s, 1H, arom.), 6.87 (d, 1H, J = 8.1 Hz, arom.), 7.09 (d, 2H, J = 8.7 Hz, arom.), 8.09 (d, 2H, J = 8.7 Hz, arom.), 11.47 (s, 1H, NH), 12.88 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.2, 46.9, 51.0, 55.3, 55.5, 55.7, 108.8, 111.3, 111.7, 114.2, 117.6, 120.8, 126.8, 131.5, 132.0, 148.2, 148.5, 164.1, 170.5 (C=O), 172.9 (C=O), 192.5 (C=O). ESI-MS *m/z* 488 $(M+H)^+$. Anal. Calcd for $C_{23}H_{21}NO_7S_2$: C, 56.66; H, 4.34; N, 2.87. Found: C, 56.64; H, 4.35; N, 2.88.

rel-(5R,6S,7R)-6-(4-Bromobenzoyl)-7-(4-methoxyphenyl) -2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3l**). Yield 56%, mp 118–120 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 4.01 (d, 1H, J = 10.2 Hz, 7-H), 4.44 (t, 1H, J = 10.2 Hz, 6-H), 4.83 (d, 1H, J = 10.2 Hz, 5-H), 7.19 (d, 2H, J = 8.4 Hz, arom.), 7.27 (d, 2H, J = 8.4 Hz, arom.), 7.64 (d, 2H, J = 8.1 Hz, arom.), 7.82 (d, 2H, J = 8.1 Hz, arom.), 11.40 (s, 1H, NH), 13.20 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1, 47.2, 50.6, 54.9, 113.8, 125.2, 128.1, 128.8, 129.3, 130.0, 130.5, 131.6, 133.5, 158.3, 170.6 (C=O), 170.7 (C=O), 193.7 (C=O). ESI-MS *m/z* 506/508 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}BrNO_5S_2$: C, 49.81; H, 3.18; N, 2.77. Found: C, 49.82; H, 3.17; N, 2.78.

rel-(5R,6S,7R)-6-Benzoyl-7-phenyl-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3m**). Yield 59%, mp 158–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.06 (d, 1H, J = 10.2 Hz, 7-H), 4.41 (t, 1H, J = 10.2 Hz, 6-H), 4.82 (d, 1H, J = 10.2 Hz, 5-H), 7.03-7.13 (m, 7H, arom.), 7.39 (t, 1H, J = 7.5 Hz, arom.), 7.58 (d, 2H, J = 7.5 Hz, arom.), 11.54 (s, 1H, NH), 12.74 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.9, 47.5, 49.4, 108.3, 118.4, 127.5, 127.8, 127.9, 128.1, 128.6, 132.5, 136.9, 138.9, 169.3 (C=O), 170.4 (C=O), 201.5 (C=O). ESI-MS *m/z* 398 $(M+H)^+$. Anal. Calcd for $C_{20}H_{15}NO_4S_2$: C, 60.44; H, 3.80; N, 3.52. Found: C, 60.42; H, 3.81; N, 3.53.

rel-(5R,6S,7R)-6-Benzoyl-7-(4-chlorophenyl)-2-oxo-3,5, 6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3n**). Yield 76%, mp 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.08 (d, 1H, J = 10.2 Hz, 7-H), 4.41 (t, 1H, J = 10.2 Hz, 6-H), 4.85 (d, 1H, J = 10.2 Hz, 5-H), 7.12 (d, 2H, J = 8.7 Hz, arom.), 7.16 (d, 2H, J = 8.7 Hz, arom.), 7.38 (t, 1H, J = 8.1 Hz, arom.), 7.49 (t, 2H, J = 8.1 Hz, arom.), 7.56 (d, 2H, J = 8.1 Hz, arom.), 11.58 (s, 1H, NH), 13.14 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.9, 48.1, 50.1, 108.4, 119.6, 128.7, 128.9, 131.4, 132.9, 133.5, 137.4, 138.9, 140.6, 170.1 (C=O), 171.2 (C=O), 202.2 (C=O). ESI-MS *m/z* 433/434 $(M+H)^+$. Anal. Calcd for $C_{20}H_{14}ClNO_4S_2$: C, 55.62; H, 3.27; N, 3.24. Found: C, 55.64; H, 3.29; N, 3.22.

rel-(5R,6S,7R)-7-(4-Bromophenyl)-6-(4-chlorobenzoyl)-2 -oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3o**). Yield 51%, mp 226–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.08 (d, 1H, J = 10.5 Hz, 7-H), 4.42 (t, 1H, J = 10.5 Hz, 6-H), 4.86 (d, 1H, J = 10.5 Hz, 5-H), 7.10 (d, 2H, J = 8.1 Hz, arom.), 7.27 (d, 2H, J = 8.1 Hz, arom.), 7.35 (d, 2H, J = 8.4 Hz, arom.), 7.64 (d, 2H, J = 8.4 Hz, arom.), 11.60 (s, 1H, NH), 13.63 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1, 47.1, 49.2, 107.3, 118.8, 120.8, 127.9, 129.9, 130.9, 131.1, 135.6, 137.6, 138.3, 169.3 (C=O), 170.33 (C=O), 200.5 (C=O). ESI-MS *m/z* 510/512/514 $(M+H)^+$. Anal. Calcd for $C_{20}H_{13}BrClNO_4S_2$: C, 47.03; H, 2.57; N, 2.74. Found: C, 47.02; H, 2.55; N, 2.73.

rel-(5R,6S,7R)-7-(4-Chlorophenyl)-6-(4-methoxybenzoyl) -2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3p**). Yield 54%, mp 200–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (t, 1H, J = 10.5 Hz, 6-H), 3.89 (s, 3H, OCH_3), 4.19 (d, 1H, J = 10.5 Hz, 7-H), 5.80 (d, 1H, J = 10.5 Hz, 5-H), 6.95 (d, 2H, J = 8.7 Hz, arom.), 7.10 (d, 2H, J = 8.7 Hz, arom.), 7.28 (d, 2H, J = 8.4 Hz, arom.), 7.41 (d, 2H, J = 8.4 Hz, arom.), 11.56 (s, 1H, NH), 13.36 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.8, 46.7, 51.1, 55.7, 107.8, 114.2, 118.2, 128.5, 130.3, 130.5, 131.6, 139.2, 141.6, 164.1, 170.6 (C=O), 172.6 (C=O), 192.4 (C=O). ESI-MS *m/z* 462/464 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}ClNO_5S_2$: C, 54.60; H, 3.49; N, 3.03. Found: C, 54.62; H, 3.47; N, 3.04.

rel-(5R,6S,7R)-6-(4-Chlorobenzoyl)-7-(4-chlorophenyl)-2 -oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3q**). Yield 63%, mp 222–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.10 (d, 1H, J = 10.2 Hz, 7-H), 4.43 (t, 1H, J = 10.2 Hz, 6-H), 4.87 (d, 1H, J = 10.2 Hz, 5-H), 7.14 (d, 2H, J = 8.7 Hz, arom.), 7.18 (d, 2H, J = 8.7 Hz, arom.), 7.35 (d, 2H, J = 8.4 Hz, arom.), 7.65 (d, 2H, J = 8.4 Hz, arom.), 11.60 (s, 1H, NH), 13.30 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1, 47.1, 49.3, 107.4, 118.8, 127.9, 128.2, 129.9, 130.6, 132.2, 135.6, 137.6, 137.9, 169.3 (C=O), 170.4 (C=O), 200.5 (C=O). ESI-MS *m/z* 466/468 $(M+H)^+$. Anal. Calcd for $C_{20}H_{13}Cl_2NO_4S_2$: C, 51.51; H, 2.81; N, 3.00. Found: C, 51.52; H, 2.83; N, 3.02.

rel-(5R,6S,7R)-6-(4-Bromobenzoyl)-7-(4-chlorophenyl)-2 -oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazole-5-carboxylic acid (**3r**). Yield 50%, mp 214–216 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.08 (d, 1H, J = 10.2 Hz, 7-H), 4.41 (t, 1H, J = 10.2 Hz, 6-H), 4.85 (d, 1H, J = 10.2 Hz, 5-H), 7.14 (d, 2H, J = 8.7 Hz, arom.), 7.38 (d, 2H, J = 8.7 Hz, arom.), 7.48 (d, 2H, J = 8.4 Hz, arom.), 7.56 (d, 2H, J = 8.4 Hz, arom.), 11.53 (s, 1H, NH), 13.58 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.0, 47.1, 49.2, 107.4, 118.7, 128.2, 130.0, 130.6, 130.9, 131.7, 132.2, 135.9, 137.9, 169.3 (C=O), 170.3 (C=O), 200.7 (C=O). ESI-MS *m/z* 510/512/514 $(M+H)^+$. Anal. Calcd for $C_{20}H_{13}BrClNO_4S_2$:

C, 47.03; H, 2.57; N, 2.74. Found: C, 47.02; H, 2.55; N, 2.73.

Pharmacology

Free radical scavenging assay

The effect of the studied compounds on DPPH radicals was estimated according to the Blois method [34]. A solution of DPPH in ethanol (150 μM, 4 mL) was mixed with a solution of a test compound in ethanol (250 μM, 0.2 mL). The reaction mixture was vortex mixed thoroughly and incubated at room temperature in the dark for 60 min. Simultaneously, a control was prepared: An solution of ascorbic acid in ethanol (0.2 mL) was mixed with a solution of DPPH in ethanol (4 mL). Reduction of absorbance at 540 nm was recorded using ethanol as blank. Free radical scavenging activity was expressed as percent of inhibition and was calculated as follow:

$$\% \text{ Inhibition} = ([A_{DPPH} - A_s]/A_{DPPH}) \times 100\%,$$

where A_{DPPH} is the absorbance of the DPPH solution and A_s is the absorbance of a test sample. Each experiment was performed in triplicate. Results were expressed as the means ± S.D.

Antimicrobial activity (agar diffusion method)

The antimicrobial activity of the synthesized compounds was determined using a method of diffusion into agar. Aliquots (20 μL) of 0.1% of the test compounds in EtOH /DMSO/water (2:1:1) were placed into wells in agar in Petri dishes with test microbes. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone of microbial growth. The plates were incubated for 24 h at 37 °C. The inhibition zone appeared after 24 h and was measured in mm around the well in each plate. The experiments were performed in triplicate, and standard deviation was calculated. These tests were carried out using isolated clinical strains of conditionally pathogenic bacterial strains: *Staphylococcus aureus*—methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains; *Escherichia coli*; *Bacillus subtilis*, and *Candida albicans*. Streptomycin and Amphotericin-B were used as reference.

The minimum inhibitory concentrations (MICs) of the compounds were carried out using the microdilution susceptibility method. Microorganism suspensions were inoculated to the corresponding wells. Plates were incubated at 36 °C for 18 h for bacteria and fungi, respectively. The MIC values were recorded as the lowest concentration of each compound in the tubes with no turbidity (i.e., no growth) of inoculated bacteria/fungi.

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