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Green synthesis of some new thiopyrano [2,3-*d*][1,3]thiazoles using lemon juice and their antibacterial activity

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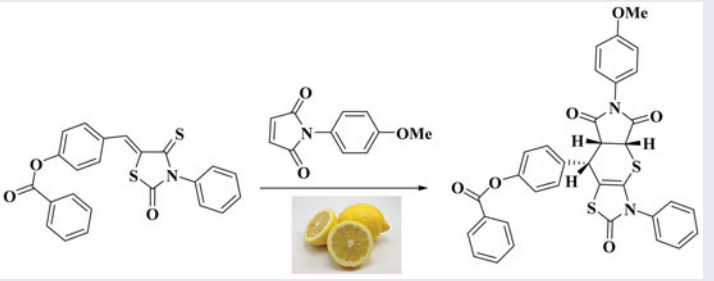
ABSTRACT

A simple green method has been developed for the synthesis of a series of new 3-phenyl-6-(substituted)-thiopyrano[2,3-*d*]thiazole-2,5,7(6H)-triones, 6-cyano-2-oxo-3-phenyl-thiopyrano[2,3-*d*]thiazoles, 3-phenyl-3,5,5a,11b-tetrahydro-2H,6H-chromeno-[4′,3′:4,5]thiopyrano[2,3-d]thiazole-2,6-dione and 5-amino-6-cyano-2-oxo-3-phenyl-3,7-dihydro-2H-thiopyrano[2,3-*d*]thiazoles *via hetero*-Diels–Alder reaction by conventional method and green method using lemon juice as natural acid. The structure of all the newly synthesized compounds was interpreted by elemental analyses and spectral data. The synthesized compounds were evaluated for their *in vitro* antibacterial activity against some pathogenic bacteria. This study is a platform for the future design of more potent antimicrobial agents.

GRAPHICAL ABSTRACT



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KEYWORDS
4-thioxo-3-phenyl-2-thiazolidinones; antibacterial activity; lemon juice; thiopyrano[2,3-*d*]thiazoles

Introduction

4-Thiazolidinones and their derivatives having valuable biological activities as antimicrobial,[1–3] antidiabetic,[4,5] anticancer,[6,7] analgesic,[8] anticonvulsant,[9,10] anti HIV,[11] antitubercular[12] and anti-inflammatory.[13,14] The improvement of the biological activity of 4-thiazolidinones based on the variation in the substituent in C-5 position of the ring and the conjugation between 5-ene exocyclic double bond with carbonyl group at position-4. Also, thiopyrano[2,3-*d*]thiazoles attracted a great attention in medicinal

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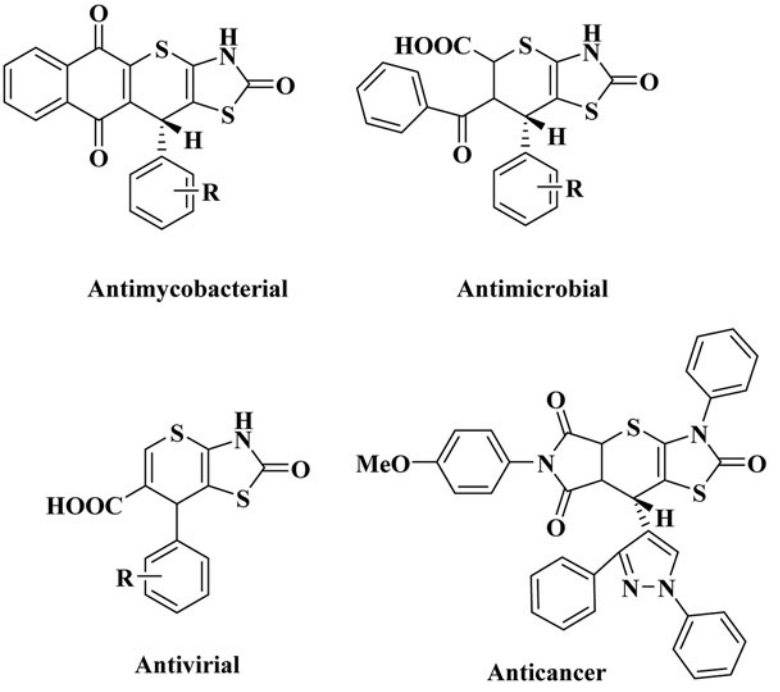
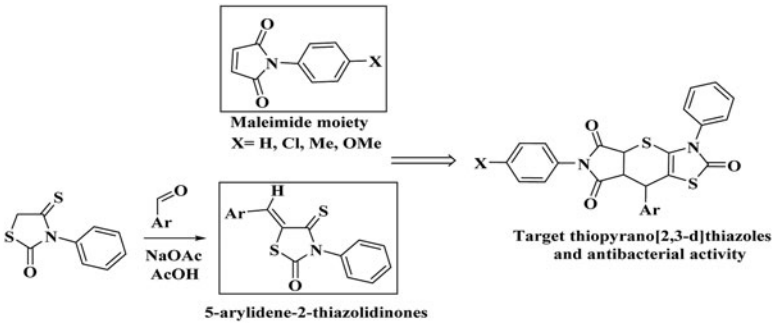


Figure 1. Background for the preparation of the target compounds.

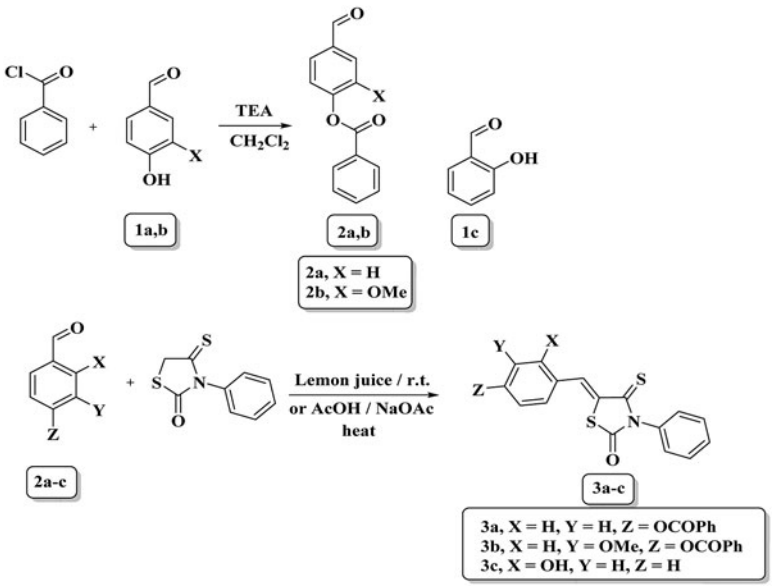
chemistry due to their diverse biological activity such as antimycobacterial,[15,16] antibacterial,[17,18] antiviral[19] and anticancer[20–22] as shown in Figure 1. Moreover, *N*-aryl maleimide derivatives displayed an efficient antimicrobial effect.[23,24] The traditional reaction conditions using in organic synthesis increases the environmental pollution posed by utility of toxic, volatile organic solvents, so in recent years, many organic researches are working on development of green methods to prepare various organic compounds by replacing hazardous acidic or basic catalyst by alternative green catalyst. Nowadays, some organic synthesis has been carried out using fruit juice of citrus lemon as a natural[25,26] because it is inexpensive, easy prepared and handling, eco-friendly and easier waste material are among the most common characteristics that make it a green catalyst. Lemon juice acts as an acidic catalyst (pH = 2–3) because percentage of citric acid (5–7%) is more than other acids.[27] An extension to our work[28–36] toward the synthesis of some new substituted thiopyrano[2,3-*d*]thiazoles *via* *hetero*-Diels–Alder reaction of interest biological activity, we synthesized some novel 5-arylmethylene-4-thioxo-2-thiazolidines and their thiopyrano[2,3-*d*]thiazoles using glacial acetic acid as conventional method and lemon juice as natural green method (Scheme 1).

Results and discussion

The target compounds, 5-arylidene-4-thioxo-2-thiazolidinones **3a–c**, were synthesized *via* Knöevenagel condensation of 3-phenyl-4-thioxo-2-thiazolidinone with



Scheme 1. Synthetic pathway for target thiopyrano[2,3-*d*][1,3]thiazoles.



Scheme 2. Synthesis of 5-arylmethylene-4-thioxo-2-thiazolidinones **3a–c**.

Table 1. Knöevenagel condensation of aromatic aldehydes 2a-c and 3-phenyl-4-thioxo-2-thiazolidinone.

Entry	X	Y	Z	Product	AcOH yield (%)	Lemon juice yield (%)
1	H	H	OCOPh	3a	99	99
2	H	OMe	OCOPh	3b	97	98
3	OH	H	H	3c	75	80

4-formylphenyl benzoate **2a**, 4-formyl-2-methoxyphenyl benzoate **2b** [prepared from alkylation for hydroxyl benzaldehyde derivatives **1a,b** with benzoyl chloride and triethylamine under stirring in methylene chloride] (Scheme 2)[37] and salicylaldehyde **1c** under stirring in lemon juice at room temperature or in refluxing glacial acetic acid with catalytic amount of fused sodium acetate (Table 1). The structure of compounds **3a–c** was proved using elemental analyses and spectral data. The IR spectrum of the isolated

Table 2. Cycloadducts thiopyrano[2,3-*d*][1,3]thiazoles **5a–o**.

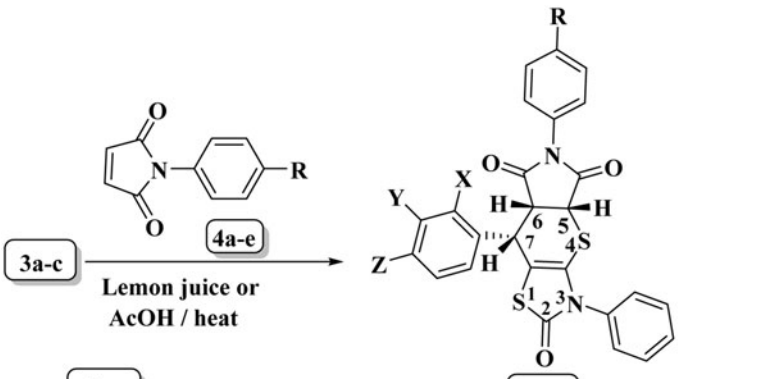
Entry	X	Y	Z	R	Product 5	AcOH yield (%)	Lemon juice yield (%)
1	H	H	OCOPh	H	a	60	68
2	H	H	OCOPh	Me	b	40	55
3	H	H	OCOPh	OMe	c	30	40
4	H	H	OCOPh	Cl	d	36	41
5	H	H	OCOPh	Br	e	45	50
6	H	OMe	OCOPh	H	f	40	46
7	H	OMe	OCOPh	Me	g	43	51
8	H	OMe	OCOPh	OMe	h	48	56
9	H	OMe	OCOPh	Cl	i	35	47
10	H	OMe	OCOPh	Br	j	40	54
11	OH	H	H	H	k	50	55
12	OH	H	H	Me	l	45	49
13	OH	H	H	OMe	m	55	61
14	OH	H	H	Cl	n	50	66
15	OH	H	H	Br	o	55	67

Table 3. Cycloadducts thiopyrano[2,3-*d*][1,3]thiazoles 7a-d.

Entry	X	R'	Product 7	AcOH yield (%)	Lemon juice yield (%)
1	H	CN	a	50	–
2	OMe	CN	b	47	–
3	H	COOEt	c	45	7
4	OMe	COOEt	d	56	9

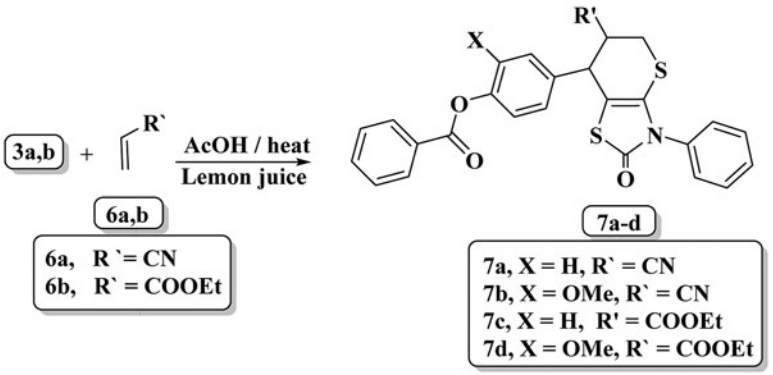
product **3a** showed carbonyl absorption bands at ν_{max} 1717 and 1584 cm^{-1}. Its ^1H NMR spectrum displayed a singlet signal at $\delta = 8.36$ ppm due to vinylic proton, in addition to the aromatic multiplet signal in the region $\delta = 7.43$-8.18 ppm. The elemental analysis together with spectral data are in agreement with the proposed structure **3** (Scheme 2).

The *hetero*-Diels–Alder reaction of **3a–c** with *N*-arylmaleimides **4a–e** in lemon juice at room temperature under stirring or in refluxing glacial acetic acid (Table 2) yielded a series of novel thiopyrano[2,3-*d*]thiazole derivatives **5a–o** (Scheme 3). The synthesized novel thiopyrano[2,3-*d*]thiazoles **5a–o** were confirmed using IR, ^1H NMR, ^{13}C NMR, MS and elemental analyses. The IR spectrum of the isolated product **5c** taken as a typical example of the prepared series showed absorption bands at $\nu_{max} = 1728$, 1682 and 1598 cm^{-1} corresponding to the three CO groups, respectively. Its ^1H NMR spectrum revealed two doublets at $\delta = 4.12$ with J values 9 and 6 Hz, assigned to H-6, another doublet signal at $\delta = 4.72$ ppm with J value 6 Hz due to H-5 and a doublet signal at $\delta = 5.18$ ppm with J value 9 Hz attributed to H-7. Also, ^{13}C NMR spectrum of **5c** showed the characteristic signals at 55.3, 164.4, 168.7, 173.5 and 173.6 ppm assigned for methoxy and carbonyl carbons, respectively. The absence of signal doubling in ^1H NMR spectra confirmed that the only one stereoisomer was present for all cycloadducts **5a–o**, and based on the values of coupling constant, the *cis*-configuration[36,38] was assigned to the cycloadducts **5** taking into account a previously studied stereochemical configuration of analog derivatives 8-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-



5	X	Y	Z	R
a	H	H	OCOPh	H
b	H	H	OCOPh	Me
c	H	H	OCOPh	OMe
d	H	H	OCOPh	Cl
e	H	H	OCOPh	Br
f	H	OMe	OCOPh	H
g	H	OMe	OCOPh	Me
h	H	OMe	OCOPh	OMe
i	H	OMe	OCOPh	Cl
j	H	OMe	OCOPh	Br
k	OH	H	H	H
l	OH	H	H	Me
m	OH	H	H	OMe
n	OH	H	H	Cl
o	OH	H	H	Br

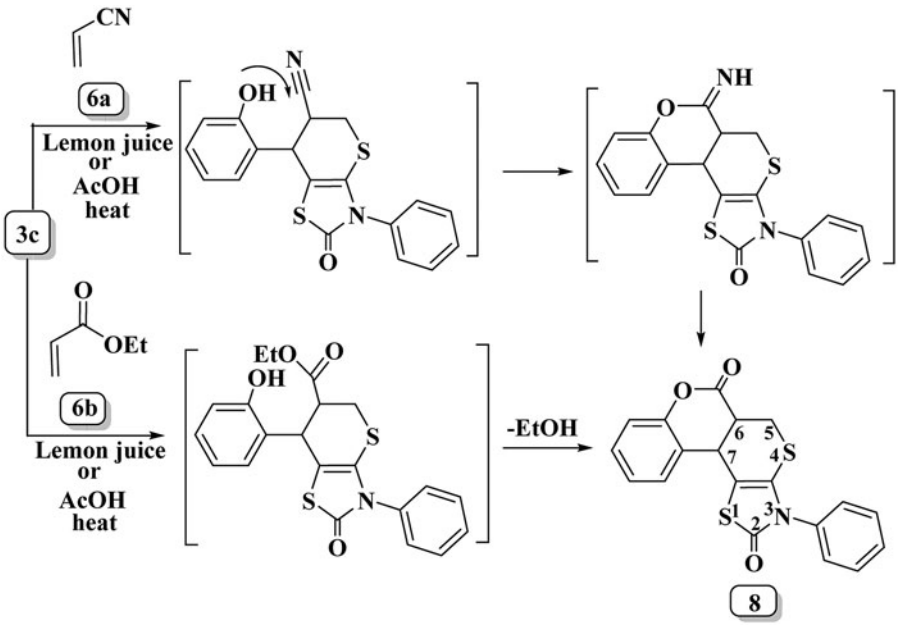
Scheme 3. Synthesis of 3-phenyl-thiopyrano[2,3-*d*]thiazole-2,5,7(6*H*)-triones **5a–o**.



Scheme 4. Synthesis of cycloadducts **7a–d**.

3,6-diphenyl-7a,8-dihydropyrrolo[3′,4′:5,6]-thiopyrano[2,3-*d*]thiazole-2,5,7(3*H*,4a*H*,6*H*)-trione, which was established using X-ray crystallography analysis.[35]

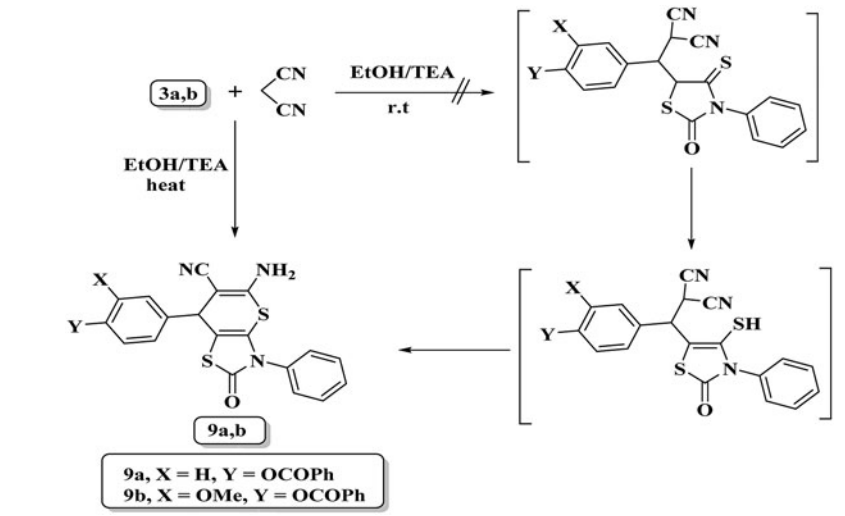
Extending to our work, the reaction of **3a,b** with acrylonitrile **6a** and ethylacrylate **6b** in refluxing glacial acetic acid (Table 3) furnished the cycloadducts **7a–d** (Scheme 4).



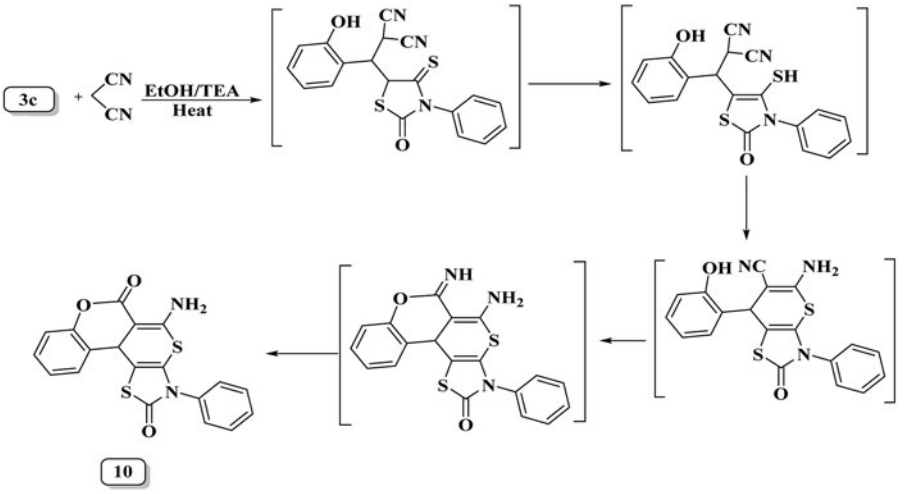
Scheme 5. Synthesis of 3-phenyl-3,5,5a,11*b*-tetrahydro-2*H*,6*H*-chromeno[4′,3′:4,5]-thiopyrano[2,3-*d*]thiazole-2,6-dione **8**.

The structure of the cycloadducts was proved using elemental analyses and spectral data. For example, the IR spectrum of **7a** showed absorption bands corresponding to cyano and carbonyl groups at $\nu_{max} = 2240$, 1745 and 1625 cm^{-1}, respectively. Its ^1H NMR spectrum revealed doublets at $\delta = 4.50$ with J value 5.4 Hz corresponding to H-7, another triplet signal at $\delta = 4.0$ ppm with J value 5.4 Hz assigned for H-6 in addition to a multiplet signal at $\delta = 3.75$–3.81. Also, ^{13}C NMR spectrum of **7a** showed the characteristic three signals at $\delta = 118.0$, 164.2 and 168.3 ppm assigned for cyano and carbonyl carbons, respectively. Also, the absence of signal doubling in ^1H NMR spectra confirmed that the only one stereoisomer was present for all cycloadducts **7a–d**. The mass spectrum together with the elemental analyses confirmed the proposed structure **7** (Scheme 4).

On the other hand, when compound **3c** reacted with acrylonitrile **6a** in lemon juice or refluxing in acetic acid furnished the cycloadduct **8** (Scheme 5). The structure of the cycloadduct **8** was established using elemental analyses and spectral data. For example, the IR spectrum of **8** showed disappearance of an absorption band corresponding to cyano group in the expected region instead two absorption bands corresponding to carbonyl groups at $\nu_{max} = 1767$ and 1671 cm^{-1}, respectively. Its ^1H NMR spectrum showed two doublet signals at $\delta = 3.47$ and 4.46 ppm with J values 3.6 and 6.0 Hz corresponding to H-5 and H-7, respectively. The collective data of cycloadduct **8** suggested the formation of **8** through 4 + 2 cycloaddition of acrylonitrile as dienophile on the exocyclic double bond of thiazolidinone ring followed by heterocyclization, *via* addition of hydroxyl group on cyano function in acrylonitrile afforded the non-isolable imino intermediate, which converted into the desired cycloadduct **8** *via* acid hydrolysis (Scheme 5). The same product was resulted by the reaction of **3c** with ethylacrylate under the same



Scheme 6. Synthesis of 5-amino-6-cyano-2-oxo-3-phenyl-3,7-dihydro-2*H*-thiopyrano[2,3-*d*]-thiazoles **9a,b**.



Scheme 7. Synthesis of 5-amino-3-phenyl-3,11b-dihydro-2*H*,6*H*-chromeno[4′,3′:4,5]-thiopyrano[2,3-*d*]thiazole-2,6-dione.

reaction conditions (mp., m.mp, TLC), which supported the formation of cycloadduct **8** (Scheme 5).

The behavior of **3a–c** toward the action of malononitrile has been investigated. Thus, the refluxing of **3a–c** with malononitrile in absolute ethanol and in the presence of few drops of triethylamine afforded products **9a–b** (Scheme 6) and **10** (Scheme 7). The reaction proceeds *via* Micheal addition of active methylene to double bond. Then tautomerism occurred through proton transfer in an intramolecular fashion from methylene group to the sulfur of thiazole ring followed by cyclization by addition of SH proton to the cyano group to yield the novel compounds **9a-b**. In case of **3c,** the reaction completed by another cyclization afforded the final product **10**.

Table 4. Zone of inhibition for synthesized compounds **3a–c**, **5a–d, 5f–l** and **5k–n** expressed in the form of mean ± standard deviation (mm).

Compound	*Escherichia coli* ATCC 3008	*Klebsiella pneumonia* ATCC 4415	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 6538	*Streptococcus mutans* ATCC 25175
3a	19.3 ± 0.6	14.3 ± 0.5	NA	NA	11.3 ± 0.5
3b	10.7 ± 0.6	12.7 ± 0.5	NA	NA	NA
3c	14.3 ± 1.5	17.6 ± 0.5	25 ± 0.5	10.5 ± 0.7	24.5 ± 0.7
5a	12.3 ± 0.5	NA	NA	NA	NA
5b	16.0 ± 1.0	16.3 ± 1.5	NA	NA	14.3 ± 0.5
5c	16.7 ± 0.6	11.3 ± 0.5	NA	NA	14.3 ± 0.6
5d	14.3 ± 0.5	14.7 ± 0.5	NA	NA	11.7 ± 1.5
5f	12.7 ± 0.6	17.7 ± 0.6	19.7 ± 0.5	14.7 ± 0.5	NA
5g	NA	NA	NA	NA	NA
5h	NA	NA	NA	10.7 ± 0.6	NA
5i	NA	10.7 ± 0.5	NA	12.3 ± 0.5	NA
5k	14.3 ± 1.5	17.6 ± 0.5	25 ± 0.5	10.5 ± 0.7	24.5 ± 0.7
5l	19.3 ± 0.6	14.3 ± 0.5	NA	NA	11.3 ± 0.5
5m	10.7 ± 0.6	12.7 ± 0.5	NA	NA	NA
5n	NA	12.6 ± 0.1	35 ± 1	NA	20 ± 1
Gentamicin	35 ± 0.6	34 ± 0.5	30 ± 0.5	–	–
Ampicilin	–	–	–	30 ± 0.5	35 ± 0.6

Zone of inhibition is expressed in the form of mean ± standard deviation (mm).
NA: No activity.
Well diameter (6 mm) and 100 µL was tested.

Antibacterial activity

The synthesized compounds **3a–c**, **5a–d, 5f–i** and **5k–n** were screened for their *in vitro* antibacterial activity against Gram-negative (*Escherichia coli* ATCC 3008, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 4415) and Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Streptococcus mutans* ATCC 25175) microorganisms (Table 4). The reference drugs are Gentamicin for Gram-negative bacteria and Ampicilin for Gram-positive bacteria. Also, the minimal inhibitory concentrations (MIC in µg/mL) or the lowest drug concentrations that stop the growth of bacteria (Table 4) were determined. The results of MIC tests against Gram-positive and Gram-negative bacteria showed that compound **3a** exhibited highest activity against *E. coli* ATCC 3008 with MIC; 15.6 µg/mL followed by compounds **5b** and **5c** with MIC; 62.5 µg/mL then compounds **5m** and **5n** with MIC; 250, 500 µg/mL, respectively. Also, compounds **3a, 3c, 5f** and **5n** have the same activity against *K. pneumonia* ATCC 4415 with MIC; 125 µg/mL followed by compounds **5b, 5c** and **5m** with MIC; 250 µg/mL. On the other hand, compounds **5f** and **5l** exhibited the same activity against *P. aeruginosa* ATCC 27853 with MIC; 125 µg/mL. Compound **5l** showed the highest activity against *S. aureus* ATCC 6538 with MIC; 250 µg/mL followed by **5m** and **5n** with MIC; 500 µg/mL. Additionally, compounds **3a, 3c, 5b, 5c, 5k** and **5l** showed selective activities against *S. mutans* ATCC 25175 whereas compound **3a** exhibited the highest activity with 31.25 µg/mL followed by compounds **5b, 5c** and **5l** MIC 62.5 µg/mL (Table 5).

Antimicrobial assay

The antimicrobial activity of synthesized compounds was determined using agar well diffusion method.[39] All the compounds were tested *in vitro* for their antibacterial activity against *S. aureus* and *S. mutans* (Gram-positive bacteria), *E. coli*, *P. aeruginosa* and

Table 5. The minimal inhibitory concentrations (MIC in μg/ml) of compounds **3a–c**, **5a–d**, **5f–l** and **5k–n**.

Compound	*Escherichia coli* ATCC 3008	*Klebsiella pneumonia* ATCC 4415	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 6538	*Streptococcus mutans* ATCC 25175
3a	15.6	125	–	–	31.25
3b	Stock	Stock	–	–	–
3c	–	125	Stock	–	250
5a	Stock	–	–	–	–
5b	62.5	250	–	–	62.5
5c	62.5	250	–	–	62.5
5d	Stock	Stock	–	Stock	Stock
5f	–	125	125	Stock	–
5g	–	–	–	–	–
5h	–	–	–	Stock	–
5i	–	Stock	–	Stock	–
5k	–	–	Stock	–	125
5l	Stock	–	125	250	62.5
5m	250	250	Stock	500	–
5n	500	125	–	500	Stock

Stock concentration 1 mg/mL.
Concentrations unit of MIC are represented as μg/mL.

klebsiella (Gram-negative bacteria) using nutrient agar medium. Ampicillin and Gentamicin were used as standard drugs for Gram-positive and Gram-negative, respectively. DMSO was used as a solvent control. The compounds were tested at a concentration of 15 mg/mL against both bacterial and fungal strains.

Method of testing

The sterilized media was poured onto the sterilized Petri dishes (20–25 mL, each petri dish) and allowed to solidify at room temperature. Microbial suspension was prepared in sterilized saline equivalent to McFarland 0.5 standard solution (1.5×10^5 CFU mL^{-1}) and its turbidity was adjusted to OD = 0.13 using spectrophotometer at 625 nm. Optimally, within 15 min after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension and was flooded on the dried agar surface then allowed to dry for 15 min with lid in place. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer. 100 μL of the solution of the tested compound was added to each well with the help of micropipette. The plates were incubated at 37 °C for 24 h in case of antibacterial activity. This experiment was carried out in triplicate and zones of inhibition were measured in millimeter scale

Methodology of MIC

For each strain, three to five isolated colonies were selected from the fresh agar plate and were transferred into a tube containing 3–4 mL of sterile broth medium. The bacterial suspension was mixed well and incubated at 35–37 °C for 2–6 h. The turbidity of the bacterial suspension should be equal to or greater than the turbidity of a McFarland Standard 0.5. After that, 1 mg of the tested compound (antimicrobial agent) was dissolved in 1 mL DMSO and two-fold serial dilution was done using broth medium. A fixed volume of the prepared bacterial inoculums was added to each tube and incubated

for at 37 °C 16–20 h. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye.[40]

Conclusion

The aim of our study was to synthesis of some new thiopyrano[2,3-*d*][1,3]thiazole derivatives from 5-arylmethylene-3-phenyl-4-thioxo-2-thiazolidinones and some dienophiles. The structures of the final cycloadducts were elucidated by elemental analyses and spectral data. Some of the newly synthesized adducts are investigated their antibacterial activity against one or more type of bacteria. 4-[(2-Oxo-3-phenyl-4-thioxothiazolidin-5-ylidene)methyl]phenyl benzoate **3a** and its cycloadducts **5b** and **5c** exhibited highest activity among other compounds, this may be due to alkylation for hydroxyl benzaldehyde that raising the antibacterial activity. On the other hand, addition of methoxy group in meta-position as in compound **3b** reduce the antibacterial activity toward Gram-positive and Gram-negative bacteria.

Experimental

Chemistry

All melting points were measured using Electrothermal (9100) apparatus and are uncorrected. The IR spectra were determined using KBr pellets on a Perkin Elmer 1430 spectrophotometer. The NMR spectra were recorded with a Varian Mercury VXR-300 NMR spectrometer at 75 and 300 MHz (^{1}C and ^{1}H NMR spectra, respectively) using DMSO-*d*6 as solvent and results are expressed as δ values. Mass spectra were taken on a Shimadzu GCMS-QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalyses Center at Cairo University and were performed on Vario EL III Elemental CHNS analyzer. Antimicrobial activity was performed at Chemistry Department at Faculty of Science in Cairo University-Giza.

Preparation of compounds (3a–c)

General procedure

Conventional method A. Solution of 3-phenyl-4-thioxo-2-thiazolidinone **1** (0.01 mol) in 10 mL of glacial acetic acid and 4-formylphenyl benzoate, 4-formyl-2-methoxyphenyl benzoate or salicylaldehyde **2a–c** (0.01 mol) with fused sodium acetate (0.015 mol) was refluxed for 2 h then left overnight at room temperature. The precipitated was filtered off, washed with water and finally dried and recrystallized from ethanol–dioxane mixture.

Preparation of lemon juice. Fresh lemon was purchased from the local market. The pieces were made using a knife and pressed in a fruit juicer to obtain the juice. Then the juice was filtered through filter paper to remove solid material and clear portion of juice was used as a catalyst.

Green method B. Solution of 3-phenyl-4-thioxo-2-thiazolidinone **1** (0.01 mol) in 3 mL of limon juice and 4-formylphenyl benzoate, 4-formyl-2-methoxyphenyl benzoate or salicylaldehyde **2a–c** (0.01 mol) was stirred at room temperature. After reaction completion as analyzed using TLC, the reaction mixture was quenched with cold water and stirred continuously until free flowing solid was obtained. The resulting solid was filtered, dried and recrystallization from ethanol-dioxane mixture.

4 *-[(2-Oxo-3-phenyl-4-thioxothiazolidin-5-ylidene)methyl]phenyl benzoate (**3a**).* Orange crystals, yield =99%, m.p 220 °C; ν_{max}/cm^{-1} (KBr) 1717 (CO), 1584 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.43–8.18 (m, 14H, Ar), 8.36 (s, 1H, CH); ^{13}C NMR (DMSO) $\delta = 120.3$, 122.9, 128.1, 128.2, 128.3, 128.7, 129.1, 129.6, 131.1, 131.7, 133.9, 135.7, 136.0, 152.0, 163.9, 193.9; m/z = 418 (M^++1, 5.43%), 417 (M^+, 18.75%), 385 (0.25%), 357 (0.25%), 312 (1.84%), 297 (1.40%), 279 (0.53%), 223 (0.47%), 181 (0.36%), 149 (0.89%), 121 (2.30%) 109 (0.50%), 191 (0.16%), 183 (0.36), 151 (0.42%), 121 (0.66%), 105 (100%), 88 (0.86%), 77 (48.34%). Anal. Calcd for $C_{23}H_{15}NO_3S_2$: C, 66.17; H, 3.62; N, 3.35; S, 15.36. Found: C, 66.35; H, 3.43; N, 3.13; S, 15.18%.

Preparation of cycloadducts 5a–o

General procedure

Conventional method A. A mixture of **3a–c** (0.01 mol) and *N*-arylmaleimides **4a–e** (0.01 mol) in glacial acetic acid (20 ml) was refluxed till decolourization took place, then it was left overnight at room temperature. The solid obtained was filtered off, and recrystallized from ethanol–dioxane mixture. The spectral data of compounds **5a–o** are shown below.

Green method B. A mixture of **3a–c** (0.01 mol) in 3 ml of lemon juice was stirred and left overnight at room temperature. After reaction completion as analyzed by TLC, the reaction mixture was quenched with cold water and stirred continuously until free-flowing solid was obtained. The resulting solid was filtered, dried and recrystallized from ethanol–dioxane mixture.

4 *-(6-(4-Methoxyphenyl)-2,5,7-trioxo-3-phenyl-2,3,4a,5,6,7,7a,8-octahydropyrrolo [3',4':5,6]thiopyrano[2,3-d]thiazol-8-yl)phenyl benzoate (**5c**).* Beige crystals, yield = 30%, m.p 220 °C; ν_{max}/cm^{-1} (KBr) 1728 (CO), 1682 (CO), 1598 (CO); ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H, OCH_3), 4.12 (dd, 1H, $J = 8.7$, 6 Hz, H-6), 4.72 (d, 1H, $J = 6$ Hz, H-5), 5.18 (d, 1H, $J = 8.7$ Hz, H-7), 6.65–6.68 (m, 2H, Ar), 6.95–7.62 (m, 14H, Ar), 7.64–8.18 (m, 2H, Ar); ^{13}C NMR (DMSO) $\delta = 55.3$, 108.3, 114.2, 114.3, 121.9, 122.9, 123.7, 127.8, 127.8, 128.7, 128.8, 129.0, 129.3, 129.4, 129.6, 130.4, 133.9, 134.2, 134.3, 150.2, 159.1, 164.4, 168.7, 173.5, 173.6; m/z = 621 (M^++1, 1.25%), 620 (M^+, 3.11%), 587 (1.76%), 560 (3.46%), 515 (0.3%), 417 (6.1%), 383 (3.1%), 296 (1.9%), 223 (0.34%), 203 (5.3%), 188 (1.8%), 134 (1.2%), 121 (2.0%), 105 (100%), 89 (0.9%), 77 (33.26%); Anal. Calcd for $C_{34}H_{24}N_2O_6S_2$: C, 65.79; H, 3.90; N, 4.51; S, 10.33. Found: C, 65.97; H, 3.73; N, 4.73; S, 10.51%.

Preparation of cycloadducts 7a–d, 8

General procedure

To each of **3a-b** (0.01 mol) in glacial acetic acid (20 mL) was added acrylonitrile **6a** or ethyl acrylate **6b** (0.01 mol). The mixture was refluxed till decolorization took place, then it was left overnight at room temperature. The solid was filtered off and recrystallized from ethanol–dioxane mixture.

4 *-(6-Cyano-2-oxo-3-phenyl-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d]thiazol-7-yl)phenyl benzoate (**7a**).* Beige crystals, yield =50%, m.p 180 °C; ν_{max}/cm^{-1} (KBr) 2240 (CN), 1745 (CO), 1625 (CO); ^{1}H NMR (DMSO-d_6) δ: 3.75–3.81 (m, 2H, H-5), 4.0 (t, 1H, $J = 5.4$ Hz, H-6), 4.5 (d, 1H, $J = 5.4$ Hz, H-7), 7.39–8.16 (m, 14H, Ar); ^{13}C NMR (DMSO) $\delta = 103.4$, 118.0, 121.7, 122.0, 124.1, 128.0, 128.7, 129.2, 129.5, 130.2, 133.8, 135.5, 150.3, 164.2, 168.3; m/z = 471 (M^++1, 2.7%), 470 (M^+, 8.7%), 417 (7.1%), 357 (1.0%), 295 (7.5%), 279 (2.1%), 251 (1.4%), 191 (2.1%), 159 (1.5%), 132 (2.6%), 105 (100%), 88 (4.3%), 77 (39.2%); Anal. Calcd for $C_{26}H_{18}N_2O_3S_2$: C, 66.36; H, 3.86; N, 5.95; S, 13.63. Found: C, 66.20; H, 3.67; N, 6.18; S, 13.81%.

3 *-Phenyl-3,5,5a,11b-tetrahydro-2H,6H-chromeno[4',3':4,5]thiopyrano[2,3-d]thiazole-2,6-dione (**8**).* Beige crystals, yield =83%, m.p 234–236 °C; ν_{max}/cm^{-1} (KBr) 3447 (NH_2), 1767 (CO), 1671 (CO); ^{1}H NMR (DMSO-d_6) δ: 3.47 (d, 2H, $J = 3.6$ Hz, H-5), 4.03 (m, 1H, H-6), 4.46 (d, 1H, $J = 6.0$ Hz, H-7), 7.16–7.53 (m, 9H, Ar); Anal. Calcd for $C_{19}H_{13}NO_3S_2$: C, 62.11; H, 3.57; N, 3.81; S, 17.45, Found: C, 62.29; H, 3.40; N, 3.61; S, 17.64%.

General procedure of 9a,b and 10

A solution of equimolecular amounts (0.01 mol) of **3a–c**, malononitrile in ethanol (100 mL) and few drops of triethylamine was refluxed for 1 h, then left at room temperature. The solid product so obtained was filtered off and crystallized from acetic acid.

4 -(5-Amino-6-cyano-2-oxo-3-phenyl-3,7-dihydro-2H-thiopyrano[2,3-d]thiazol-7-yl)-2-methoxyphenyl benzoate (9b)

Beige crystals, yield =39%, m.p 200 °C; ν_{max}/cm^{-1} (KBr) 3452 (NH_2), 2243 (CN), 1680 (CO), 1595 (CO); ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H, OCH_3), 4.46 (s, 1H, H-7), 6.92–8.14 (m, 15H, Ar and NH_2); ^{13}C NMR (DMSO) $\delta = 55.8$, 72.1, 105.7, 111.8, 117.3, 118.5, 119.2, 123.3, 128.1, 128.6, 128.8, 129.6, 129.66, 129.7, 133.6, 133.8, 138.7, 141.2, 150.9, 151.2; Anal. Calcd for $C_{27}H_{19}N_3O_4S_2$: C, 63.14; H, 3.73; N, 8.18; S, 12.48, Found: C, 63.33; H, 3.56; N, 8.41; S, 12.31%.

5 -Amino-3-phenyl-3,11b-dihydro-2H,6H-chromeno[4',3':4,5]thiopyrano[2,3-d]thiazole-2,6-dione (10)

Beige crystals, yield =43%, m.p 280 °C; ν_{max}/cm^{-1} (KBr) 3447 (NH_2), 1767 (CO), 1671 (CO); ^1H NMR (DMSO-d_6) δ: 4.40 (s, 1H, H-7), 7.08–7.61 (m, 11H, Ar and NH_2); Anal. Calcd for $C_{19}H_{12}N_2O_3S_2$: C, 59.99; H, 3.18; N, 7.36; S, 16.85. Found: C, 59.81; H, 3.35; N, 7.59; S, 16.68%.

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