

Article

Microwave Assisted Synthesis of Some New Thiazolopyrimidine, Thiazolodipyrimidine and Thiazolopyrimidothiazolopyrimidine Derivatives with Potential Antioxidant and Antimicrobial Activity

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Abstract: Biginelli reaction of ethyl acetoacetate, thiourea and the appropriate aromatic aldehyde was used to produce ethyl 4-aryl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates, that reacted with bromomalononitrile to give ethyl 3-amino-5-aryl-2-cyano-7-methyl-5*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylates rather than the isomeric 7*H*-thiazolo[3,2-*a*]pyrimidines. Thiazolopyrimidine derivatives reacted with carbon disulphide to yield ethyl 9-aryl-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1*H*-thiazolo[3,2-*a*:4,5-*d'*]dipyrimidine-8-carboxylates, that reacted with phenacyl bromide to produce ethyl 8-methyl-10-(4-methoxyphenyl)-3-substituted-5-thioxo-2(un)substituted-10*H*-thiazolo[3",2":1',2']pyrimido[4',5':4,5]thiazolo[3,2-*a*]pyrimidine-9-carboxylates. The aforementioned reactions were carried out using both conventional chemical methods and with the assistance of microwave irradiation. Comparison between both methods showed that the microwave assisted method is preferable because of the time reduction and yield improvements achieved. The new compounds were tested for their biological activity as antioxidants, antibacterial or antifungal agents. Some of the new compounds were found to have moderate to good antioxidant and antimicrobial activities.

Keywords: biginelli reaction; 2-thioxopyrimidines; thiazolopyrimidines; thiazolo-dipyrimidines; thiazolopyrimidothiazolopyrimidines; antioxidant activity; antimicrobial activity

1. Introduction

Thiazolopyrimidines have been of interest due to their ability to inhibit 2-methylerythritol-2,4-cyclodiphosphate synthase [1]. They have been also used as analgesic and antiparkinsonian agents [2], modulators of Transient Receptor Potential Vanilloid-receptor 1 (TRPV1) [3], anticancer agents [4–6], pesticides [7], phosphate inhibitors [8,9], acetylcholinesterase inhibitors [10] and antimicrobial substances [11–13].

The microwave technique has several advantages over conventional methods of synthesis. Reduced reaction times [14–17], less effects on the environment and better reaction yields are some of the common advantages of using microwave irradiation. In the present research, we used both the microwave technique as well as conventional methods to prepare some thiazolopyrimidine, thiazolodipyrimidine and thiazolopyrimidothiazolopyrimidines derivatives with expected biological activity.

2. Results and Discussion

2.1. Chemistry

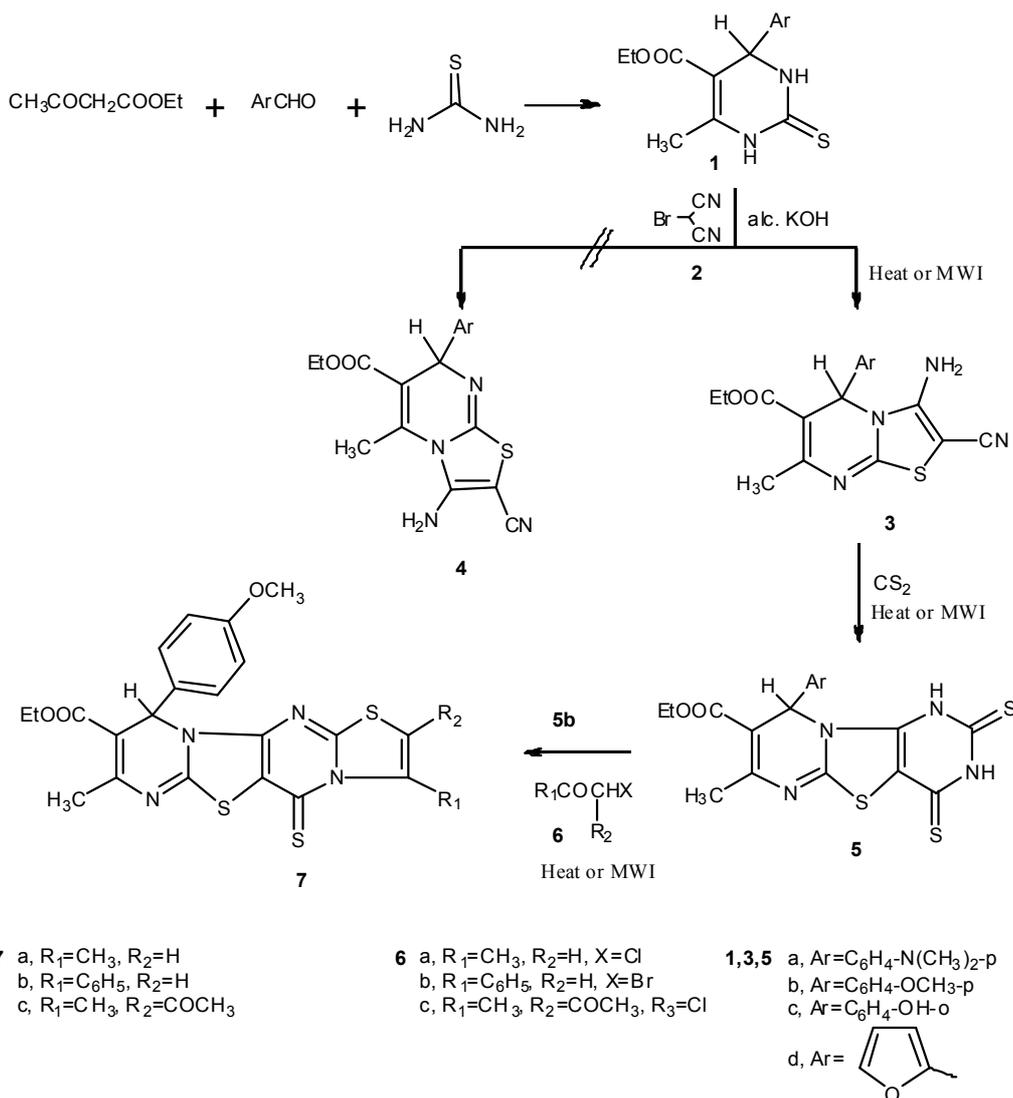
Prompted by the aforesaid biological and medicinal activities, samples of differently substituted thiazolopyrimidines and thiazolodipyrimidines were synthesized, using both conventional chemical methods and microwave irradiation assistance. The reaction of the precursors, ethyl 4-aryl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **1a–d**, with some bifunctional reagents seems to be a facile and convenient route for the synthesis of such targets. The newly synthesized compounds were tested for their antioxidant and antimicrobial activities.

The precursor pyrimidine derivatives **1a–d** were prepared by the acid catalyzed condensation of ternary mixtures of aromatic aldehydes, ethyl acetoacetate and thiourea in ethanol containing a catalytic amount of hydrochloric acid, commonly known as Biginelli reaction [18–20] (Scheme 1). Compounds **1a–d**, prepared by Biginelli's method, showed correct values of elemental analyses, as well as compatible spectroscopic data.

Treating each of **1a–d** with bromomalononitrile (**2**) in an ethanol solution containing potassium hydroxide yielded in each case a single product which could in principle be formulated to be either the 5*H*-thiazolo[3,2-*a*]pyrimidine structure **3** or the isomeric 7*H*-thiazolo[3,2-*a*]pyrimidine structure **4** (Scheme 1). Our preference for structure **3** over structure **4** was firstly based on comparison of the ¹H-NMR spectral data for compounds **1** and **3**. Thus, the ¹H-NMR spectrum of **3b** showed, in addition to the ethyl ester, methoxy, aromatic and NH₂ proton signals, a singlet (3H) at δ 2.30 ppm assigned to the CH₃ protons and a singlet (1H) at δ 6.31 assigned to the pyrimidine H-5. The appearance of the CH₃ proton signal of **3b** in the same position as that for the CH₃ proton signal in **1b**, and also the downfield shift for the pyrimidine H-5 in **3b** compared with the pyrimidine H-4 in **1b**, which appeared at δ = 5.12 ppm, indicates that the moiety around H-5 in **3b** differs from that around

H-4 in **1b**. Also, the moiety around CH₃ at C-7 in **3b** is almost similar to that around CH₃ at C-6 in **1b**. Consequently, structure **3** could be tentatively assigned for the reaction products. Structure **4** would be expected to show different δ values for the CH₃ groups in **4b** and **1b**, and similar δ values for H-7 in **4b** and H-4 in **1b**. More conclusive evidence for structure **3** was based on carrying out a NOE experiment on compound **3b**.

Scheme 1. Synthesis of pyrimidinethiones, thiazolopyrimidines, thiazolidiprimidines and thiazolopyrimidothiazolopyrimidines.



Structure **4**, having a CH₃ group at C-5 and NH₂ at C-3 in close proximity, would show a change in CH₃ position signal due to NOE. Actually, upon performing the NOE experiment, the position of the CH₃ group was not affected, which indicates that CH₃ and NH₂ are not close to each other, thus suggesting structure **3** for the reaction product.

Compounds **3**, as typical enaminonitriles, could be used as precursors for the preparation of thiazolidiprimidines. Thus, heating under reflux a mixture of each of **3a–d** with an excess of carbon disulphide yielded, in each case, the corresponding 9-aryl-2,4-dithioxo-7-methylthiazolo[3,2-a:4,5-d]diprimidine-8-carboxylate **5a–d** (Scheme 1).

Finally, compound **5b** reacted with α -halocarbonyl compounds, namely chloroacetone, phenacyl bromide and 3-chloropentane-2,4-dione (compounds **6a–c**), respectively, by heating in ethanolic potassium hydroxide solution to produce the respective thiazolo[3",2":1',2']pyrimido[4',5':4,5]thiazolo-[3,2-*a*]pyrimidine-9-carboxylate derivatives **7a–c** (Scheme 1). Besides giving correct elemental analyses and compatible spectral data (see the Experimental), structure **7** was assigned to the reaction products based on the behaviour of a similar structure reported in literature by Hafez *et al.* [21].

Our research group has recently [22–25] been interested in performing synthesis of some heterocyclic compounds under environmentally friendly, time saving microwave-assisted conditions. Accordingly, we re-synthesized the previously described compounds **1a–d**, **3a–d**, **5a–d**, and **7a–c** under microwave conditions, aiming to increase reaction yields and reduce the reaction times. The results of these preparations indicated that reaction yields were increased by 17–23% compared to the conventional conditions. Reaction times were also significantly reduced. Table 1 summarizes the benefits of using microwave conditions for the synthesis of the above-mentioned compounds.

Table 1. Comparison between traditional methods and microwave assisted methods of synthesis of compounds **1a–d**, **3a–d**, **5a–d**, and **7a–c**.

Compound no.	Reaction Yield %		Reaction Time	
	Microwave	Conventional Method	Microwave	Conventional Method
1a	82	55	5 min	3 h
1b	87	58	5 min	3 h
1c	70	42	5 min	3 h
1d	62	35	5 min	3 h
3a	81	56	10 min	Overnight
3b	85	48	10 min	Overnight
3c	85	53	10 min	Overnight
3d	68	40	10 min	Overnight
5a	82	50	15 min	8 h
5b	80	53	15 min	8 h
5c	79	44	15 min	8 h
5d	69	37	15 min	8 h
7a	74	43	5 min	3 h
7b	88	52	5 min	3 h
7c	83	58	5 min	3 h

2.2. Biological Evaluation

2.2.1. Antioxidant Screening

The newly synthesized compounds were tested for antioxidant activity as reflected in their ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rat erythrocyte hemolysis. The pro-oxidant activities of the aforementioned compounds were assessed by their effects on bleomycin-induced DNA damage. Table 2 shows the antioxidant assays by erythrocyte hemolysis, which reveals that compounds **3a** and **3b** showed interesting antioxidant activity in the lipid peroxidation assays and considerable inhibitory activity in the hemolysis assay. Compounds **3c**, **3d**, **7a**, **7b** and **7c** showed

moderate antioxidant and inhibitory activity. Table 3 shows the antioxidant assay by ABTS method. Compounds **3a**, **3b**, **3c** and **3d** showed potent antioxidant activity.

Table 2. Antioxidant assays by erythrocyte hemolysis ($A/B \times 100$).

Compounds	Absorbance of samples (A)	Hemolysis (%)
Complete hemolysis with distilled water (B)	0.660	-
Ascorbic acid	0.026	3.93
1a	0.082	10.33
1b	0.075	9.12
1c	0.090	13.01
1d	0.092	14.12
3a	0.035	5.22
3b	0.031	4.68
3c	0.045	6.92
3d	0.051	8.02
5a	0.115	21.60
5b	0.112	19.25
5c	0.132	24.07
5d	0.130	23.12
7a	0.042	6.36
7b	0.045	6.81
7c	0.043	6.51

Table 3. Antioxidant assays by ABTS method [$Abs. (control) - Abs.(test)/Abs.(control) \times 100$].

Compounds	Absorbance of sample	Inhibition (%)
ABTS control	0.54	0
Ascorbic acid	0.06	88.8
1a	0.20	63.0
1b	0.23	57.4
1c	0.29	46.3
1d	0.28	48.1
3a	0.10	81.5
3b	0.12	77.7
3c	0.15	72.2
3d	0.13	75.9
5a	0.45	16.6
5b	0.42	22.2
5c	0.48	11.1
5d	0.43	20.3
7a	0.17	68.5
7b	0.19	64.8
7c	0.16	70.3

All compounds have been tested on bleomycin-dependent DNA damage. The results, shown in Table 4, indicate that compounds **3a–d** may have some protective activity towards DNA from the damage induced by bleomycin.

Table 4. Assays for bleomycin-dependent DNA damage.

Compound	Absorbance of Samples
Ascorbic acid	0.020
3a	0.026
3b	0.029
3c	0.037
3d	0.033

2.2.2. Antimicrobial Evaluation

The newly synthesized heterocyclic compounds listed in Table 4 were tested for their antimicrobial activity against the following microorganisms: *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis*, *Streptococcus lactis*, *Aspergillus niger*, *Penicillium sp.* and *Candida albicans*. The preliminary screening of the investigated compounds was performed using the filter paper disc-diffusion method. The most active compounds were **1a**, **1b**, **3a**, **3b**, **5a**, and **5b**, which showed moderate to slight inhibitory action towards the microorganisms. The rest of compounds showed slight to no sensitivity at all to the tested organisms, and the results are summarized in Table 5.

Table 5. Antimicrobial activities of the newly synthesized compounds.

Compd. No.	Inhibition zone (mm)						
	Gram-negative		Gram-positive		Fungi Yeast		
	<i>E. coli</i>	<i>P. putida</i>	<i>B. subtilis</i>	<i>S. lactis</i>	<i>A. niger</i>	<i>P. sp.</i>	<i>C. albicans</i>
1a	12	8	6	8	5	5	0
1b	14	9	6	7	4	2	0
1c	6	3	0	0	2	2	0
1d	3	2	0	0	0	0	0
3a	15	11	9	6	7	5	0
3b	12	7	7	5	7	5	0
3c	0	0	0	0	0	0	0
3d	2	2	0	0	0	0	0
5a	10	7	8	6	4	3	
5b	11	8	9	6	5	2	
5c	4	2	0	0	0	0	0
5d	3	0	0	0	0	0	0
7a	0	0	0	0	0	0	0
7b	0	0	0	0	0	0	0
7c	0	0	0	0	0	0	0
Chloram-phenicol [®]	22	21	18	19	20	12	0
Ampicillin [®]	24	20	19	22	24	14	14

E. coli = *Escherichia coli*; *P. putida* = *Pseudomonas putida*; *B. subtilis* = *Bacillus subtilis*; *S. lactis* = *Streptococcus lactis*; *A. niger* = *Aspergillus niger*; *P. sp.* = *Penicillium sp.*; *C. albicans* = *Candida albicans*; The sensitivity of microorganisms to the tested compounds is identified in the following manner *: Highly sensitive = Inhibition zone: 15–20 mm; Moderately sensitive = Inhibition zone: 10–15 mm; Slightly sensitive = Inhibition zone: 1–10 mm; Not sensitive = Inhibition zone: 0 mm; * each result represents the average of triplicate readings.

3. Experimental

3.1. General

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a Shimadzu FTIR-8201PC Spectrophotometer. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on a Varian Mercury 300 MHz and a Varian Gemini 200 MHz spectrometers using TMS as an internal standard and $\text{DMSO-}d_6$, and as a solvent. Chemical shifts were expressed as δ (ppm) units. Mass spectra were recorded on a Shimadzu GCMS-QP1000EX instrument using an inlet type sample injection at 70 eV. The Microanalytical Center of Cairo University performed the microanalyses. Microwave reactions were performed with a Millstone Organic Synthesis Unit (MicroSYNTH with touch control terminal) with a continuous focused microwave power delivery system in a pressure glass vessel (10 mL) sealed with a septum under magnetic stirring. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control under the reaction vessel, and control of the pressure was performed with a pressure sensor connected to the septum of the vessel.

3.1.1. Ethyl 4-aryl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **1a–d**

Method A: A solution of thiourea (0.76 g, 0.01 mol), ethyl acetoacetate (1.30 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in ethanol (50 mL) in the presence of conc. HCl (5 mL) was heated under reflux for 3 h. The reaction mixture was then allowed to stand at room temperature overnight whereby the solid precipitate so-formed was collected by filtration, washed with ethanol and crystallized from ethanol.

Method B: The same reactants of *Method A* were heated in microwave oven at 140 °C for 5 min. The reaction mixture was treated in a similar manner to *Method A* to obtain compounds **1a–d**.

Ethyl 4-(4-(dimethylamino)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1a) was obtained as pale green crystals, m.p. 201 °C. $^1\text{H-NMR}$: δ (ppm) 1.10 (t, 3H, CH_3 , $J = 4$ Hz), 2.26 (s, 3H, CH_3), 2.87 (s, 6H, $\text{N}(\text{CH}_3)_2$), 3.97 (q, 2H, CH_2 , $J = 4$ Hz), 5.02 (s, 1H, pyrimidine H-4), 6.65 (d, 2H, Ar-H, $J = 5$ Hz), 7.00 (d, 2H, Ar-H, $J = 5$ Hz), 9.55 (s, 1H, NH, D_2O exchangeable) and 10.23 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$: δ (ppm) 14.1 (CH_3), 17.2 (CH_3), 53.5 (pyrimidine C-4), 59.5 ($\text{N}(\text{CH}_3)_2$), 67.3 (CH_2), 101.2, 112.2, 127.1, 131.2, 144.4, 150.0 (aromatic carbons + pyrimidine C-5 and C-6), 165.3 (C=S) and 173.8 (C=O). IR (KBr) ν : 3268, 3188 (NH), 1718 (C=O), 1605, 1500 cm^{-1} (Aromatic C=C). MS (70 eV): (M^+) m/z 319 (11.2%). Anal. Calcd. for $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}_2\text{S}$ (319.42): C(60.16%), H(6.63%), N(13.16%), S(10.04); Found: C(60.3%), H(6.8%), N(13.4%), S(10.0%).

Ethyl 4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1b) was obtained as yellow crystals, m.p. 144 °C. $^1\text{H-NMR}$: δ (ppm) 1.22 (t, 3H, CH_3 , $J = 4$ Hz), 2.26 (s, 3H, CH_3), 3.25 (s, 3H, OCH_3), 3.95 (q, 2H, CH_2 , $J = 4$ Hz), 5.12 (s, 1H, pyrimidine H-4), 6.81 (d, 2H, Ar-H, $J = 6$ Hz), 7.11 (d, 2H, Ar-H, $J = 6$ Hz), 9.50 (s, 1H, NH, D_2O exchangeable) and 10.18 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$: δ (ppm) 14.0 (CH_3), 17.4 (CH_3), 53.5 (pyrimidine C-4), 63.9 (OCH_3), 68.8 (CH_2), 103.4, 113.1, 129.7, 134.2, 145.9 153.2 (aromatic carbons + pyrimidine C-5 and C-6),

165.6 (C=S) and 174.0 (C=O). IR (KBr) ν : 3272, 3185 (NH), 1718 (C=O), 1603, 1506 cm^{-1} (Aromatic C=C). MS (70 eV): (M^+) m/z 306 (8.5%). Anal. Calcd. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ (306.38): C(58.80%), H(5.92%), N(9.14%), S(10.47); Found: C(59.0%), H(5.9%), N(9.3%), S(10.7%).

Ethyl 4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1c) was obtained as beige crystals, m.p. 235 °C. $^1\text{H-NMR}$: δ (ppm) 1.18 (t, 3H, CH_3 , $J = 4$ Hz), 2.19 (s, 3H, CH_3), 3.95 (q, 2H, CH_2 , $J = 4$ Hz), 5.10 (s, 1H, pyrimidine H-4), 6.73–7.07 (m, 4H, Ar-H), 8.40 (s, 1H, OH, D_2O exchangeable), 9.55 (s, 1H, NH, D_2O exchangeable) and 10.12 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$: δ (ppm) 13.5 (CH_3), 16.8 (CH_3), 53.5 (pyrimidine C-4), 67.6 (CH_2), 102.7, 112.6, 115.3, 122.0, 127.7, 131.9, 144.6, 150.9 (aromatic carbons + pyrimidine C-5 and C-6), 165.2 (C=S) and 172.6 (C=O). IR (KBr) ν : 3280–3080 (broad, OH+NH), 1722(C=O), 1600, 1501 cm^{-1} (Aromatic C=C). MS (70 eV): (M^+) m/z 292 (14.1%). Anal. Calcd. for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$ (292.09): C(57.52%), H(5.52%), N(9.58%), S(10.97); Found: C(57.7%), H(5.8%), N(9.5%), S(11.1%).

Ethyl 4-(furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1d) was obtained as brown crystals, m.p. 176 °C. $^1\text{H-NMR}$: δ (ppm) 1.25 (t, 3H, CH_3 , $J = 4$ Hz), 2.22 (s, 3H, CH_3), 4.20 (q, 2H, CH_2 , $J = 4$ Hz), 5.23 (s, 1H, pyrimidine H-4), 6.44 (d, 1H, furan-H), 6.56 (m, 1H, furan-H), 7.41 (d, 1H, furan-H), 9.34 (s, 1H, NH, D_2O exchangeable) and 10.20 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$: δ (ppm) 14.3 (CH_3), 17.5 (CH_3), 57.5 (pyrimidine C-4), 67.2 (CH_2), 106.2, 110.9, 143.1, 145.3, 151.0, 152.9 (furan carbons + pyrimidine C-5 and C-6), 165.8 (C=S) and 173.0 (C=O). IR (KBr) ν : 3275, 3183 (NH), 1720 (C=O), 1607, 1500 cm^{-1} (aromatic C=C). MS (70 eV): (M^+) m/z 266 (6.3%). Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_3\text{S}$ (266.32): C(54.12%), H(5.30%), N(10.52%), S(12.04); Found: C(54.1%), H(5.4%), N(10.4%), S(12.0%).

3.1.2. Ethyl 3-amino-5-aryl-2-cyano-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylates (3a–d)

Method A: To a warm ethanolic potassium hydroxide solution [prepared by dissolving KOH (0.56 g, 0.01 mol) in ethanol (50 mL)] of each of **1a–d** (0.01 mol), bromomalononitrile **2** (1.45 g, 0.01 mol) was added portion-wise with stirring. The reaction mixture was then left overnight at room temperature, whereby the solid product that separated upon dilution with water was filtered off and crystallized from the proper solvent.

Method B: The same reactants of method A were heated at 140 °C in microwave oven for 10 min. The reaction mixture was treated in a similar manner to method A to obtain compounds **3a–d**.

Ethyl 3-amino-2-cyano-5-(4-(dimethylamino)phenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (3a) was crystallized from dil. dioxane as yellowish green crystals, m.p. 218 °C. $^1\text{H-NMR}$: δ (ppm) 1.35 (t, 3H, CH_3 , $J = 4$ Hz), 2.34 (s, 3H, CH_3), 2.95 (s, 6H, $\text{N}(\text{CH}_3)_2$), 4.15 (q, 2H, CH_2 , $J = 4$ Hz), 6.22 (s, 1H, pyrimidine H-5), 6.60 (d, 2H, Ar-H, $J = 5$ Hz), 6.97 (d, 2H, Ar-H, $J = 5$ Hz) and 8.78 (s, 2H, NH_2 , D_2O exchangeable). $^{13}\text{C-NMR}$: δ (ppm) 14.5 (CH_3), 17.3 (CH_3), 58.8 (pyrimidine C-5), 59.7 ($\text{N}(\text{CH}_3)_2$), 68.4 (CH_2), 107.3 (CN), 112.2, 117.3, 127.1, 132.2, 149.4, 154.6, 157.3, 158.1, 158.9 (aromatic carbons + pyrimidine C-6 and C-7, C-8a + thiazole C-2, C-3), and 171.6 (C=O). IR (KBr) ν : 3310, 3244 (NH_2), 2217 (CN), 1724 (C=O), 1605, 1500 cm^{-1} (aromatic C=C). MS

(70 eV): (M^+) m/z 383 (7.3%). Anal. Calcd. for $C_{19}H_{21}N_5O_2S$ (383.14): C(59.51%), H(5.52%), N(18.26%), S(8.36); Found: C(59.6%), H(5.8%), N(18.3%), S(8.3%).

Ethyl 3-amino-2-cyano-5-(4-methoxyphenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (3b) was crystallized from ethanol as yellow crystals, m.p. 220 °C. 1H -NMR: δ (ppm) 1.30 (t, 3H, CH_3 , $J = 4$ Hz), 2.30 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.18 (q, 2H, CH_2 , $J = 4$ Hz), 6.31 (s, 1H, pyrimidine H-5), 6.80 (d, 2H, Ar-H, $J = 6$ Hz), 7.15 (d, 2H, Ar-H, $J = 6$ Hz), and 8.40 (s, 2H, NH_2 , D_2O exchangeable). ^{13}C -NMR: δ (ppm) 14.8 (CH_3), 17.4 (CH_3), 59.6 (pyrimidine C-5), 62.1 (OCH_3), 67.4 (CH_2), 108.1 (CN), 113.1, 118.8, 129.0, 134.1, 151.4, 155.1, 157.8, 158.7, 159.3 (aromatic carbons + pyrimidine C-6 and C-7, C-8a + thiazole C-2, C-3), and 171.0 (C=O). IR (KBr) ν : 3300, 3230 (NH_2), 2210 (CN), 1720 (C=O), 1605, 1500 cm^{-1} (aromatic C=C). MS (70 eV): (M^+) m/z 370 (8.1%). Anal. Calcd. for $C_{18}H_{18}N_4O_3S$ (370.43): C(58.36%), H(4.90%), N(15.12%), S(8.66); Found: C(58.5%), H(4.8%), N(15.3%), S(8.5%).

Ethyl 3-amino-2-cyano-5-(2-hydroxyphenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (3c) was crystallized from ethanol as yellow crystals, m.p. 277 °C. 1H -NMR: δ (ppm) 1.32 (t, 3H, CH_3 , $J = 4$ Hz), 2.34 (s, 3H, CH_3), 4.05 (q, 2H, CH_2 , $J = 4$ Hz), 6.24 (s, 1H, pyrimidine H-5), 6.80–7.18 (m, 4H, Ar-H), 8.15 (s, 1H, OH, D_2O exchangeable) and 8.53 (s, 2H, NH_2 , D_2O exchangeable). ^{13}C -NMR: δ (ppm) 14.3 (CH_3), 17.1 (CH_3), 58.8 (pyrimidine C-5), 67.1 (CH_2), 107.3 (CN), 112.3, 116.1, 122.1, 126.3, 131.3, 137.1, 152.1, 157.1, 157.9, 158.8, 159.5 (aromatic carbons + pyrimidine C-6 and C-7, C-8a + thiazole C-2, C-3), and 173.0 (C=O). IR (KBr) ν : 3320–3118 (broad, OH+ NH_2), 2210 (CN), 1716 (C=O), 1600, 1500 cm^{-1} (aromatic C=C). MS (70 eV): (M^+) m/z 356 (12.5%). Anal. Calcd. for $C_{17}H_{16}N_4O_3S$ (356.40): C(57.29%), H(4.52%), N(15.72%), S(9.00); Found: C(57.5%), H(4.6%), N(15.6%), S(9.1%).

Ethyl 3-amino-2-cyano-5-(furan-2-yl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (3d) was crystallized from dioxane as brown crystals, m.p. 345 °C. 1H -NMR: δ (ppm) 1.27 (t, 3H, CH_3 , $J = 4$ Hz), 2.21 (s, 3H, CH_3), 4.25 (q, 2H, CH_2 , $J = 4$ Hz), 6.11 (s, 1H, pyrimidine H-5), 6.45 (d, 1H, furan-H), 6.61 (m, 1H, furan-H), 7.43 (d, 1H, furan-H) and 8.87 (s, 2H, NH_2 , D_2O exchangeable). ^{13}C -NMR: δ (ppm) 14.7 (CH_3), 17.5 (CH_3), 62.3 (pyrimidine C-5), 67.4 (CH_2), 107.1 (CN), 112.6, 114.1, 127.3, 135.1, 139.6, 143.1, 145.3, 151.0 152.9 (furan carbons + pyrimidine C-6 and C-7, C-8a + thiazole C-2, C-3) and 173.3 (C=O). IR (KBr) ν : 3310, 3244 (NH_2), 2210 (CN), 1722 (C=O), 1605, 1510 cm^{-1} (aromatic C=C). MS (70 eV): (M^+) m/z 330 (4.8%). Anal. Calcd. for $C_{15}H_{14}N_4O_3S$ (330.36): C(54.53%), H(4.27%), N(16.96%), S(9.71); Found: C(54.4%), H(4.4%), N(16.7%), S(9.8%).

3.1.3. Ethyl 9-aryl-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1H-thiazolo[3,2-a:4,5-d']dipyrimidine-8-carboxylates **5a–d**

Method A: Each of compounds **3a–d** (0.01 mol) was heated under reflux with an excess of carbon disulphide (10 mL) for 8 h. The reaction mixture was then cooled, and the solid that precipitated was filtered at the pump and crystallized from the proper solvent.

Method B: The same reactants of method A were heated at 140 °C in microwave oven for 15 min. The reaction mixture was treated in a similar manner to method A to obtain compounds **5a–d**.

Ethyl 9-(4-(dimethylamino)phenyl)-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1H-thiazolo-[3,2-a:4,5-d']dipyrimidine-8-carboxylate (5a) was crystallized from dilute ethanol as grey crystals, m.p. 248 °C. ¹H-NMR: δ (ppm) 1.10 (t, 3H, CH₃, *J* = 4 Hz), 2.26 (s, 3H, CH₃), 2.84 (s, 6H, N(CH₃)₂), 3.95 (q, 2H, CH₂, *J* = 4 Hz), 5.88 (s, 1H, pyrimidine H-9), 6.60 (d, 2H, Ar-H, *J* = 5 Hz), 6.95 (d, 2H, Ar-H, *J* = 5 Hz), 11.35 (s, 1H, NH, D₂O exchangeable) and 12.12 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR: δ (ppm) 14.2 (CH₃), 17.8 (CH₃), 53.5 (pyrimidine C-9), 59.6 (N(CH₃)₂), 67.3 (CH₂), 109.2, 111.3, 127.1, 132.2, 148.2, 154.3, 156.2, 157.1, 161.9 (aromatic carbons + pyrimidine C-5a, C-7, C-8 + thiazole C-4a, C-10a), 171.3 (C=S), 175.1 (C=O) and 181.4 (C=S). IR (KBr) ν: 3305, 3200 (NH₂), 1712 (C=O), 1605, 1500 cm⁻¹ (aromatic C=C). MS (70 eV): (M⁺) *m/z* 459 (3.2%). Anal. Calcd. for C₂₀H₂₁N₅O₂S₃ (459.61): C(52.26%), H(4.61%), N(15.24%), S(20.93); Found: C(52.3%), H(4.7%), N(15.5%), S(20.8%).

Ethyl 9-(4-methoxyphenyl)-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1H-thiazolo[3,2-a:4,5-d']dipyrimidine-8-carboxylate (5b) was crystallized from ethanol as beige crystals, m.p. 243 °C. ¹H-NMR: δ (ppm) 1.13 (t, 3H, CH₃, *J* = 4 Hz), 2.32 (s, 3H, CH₃), 3.22 (s, 3H, OCH₃), 4.10 (q, 2H, CH₂, *J* = 4 Hz), 5.95 (s, 1H, pyrimidine H-9), 6.80 (d, 2H, Ar-H, *J* = 6 Hz), 7.12 (d, 2H, Ar-H, *J* = 6 Hz), 11.30 (s, 1H, NH, D₂O exchangeable) and 12.10 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR: δ (ppm) 14.1 (CH₃), 18.6 (CH₃), 61.0 (pyrimidine C-9), 63.2 (OCH₃), 67.3 (CH₂), 110.4, 114.6, 130.0, 133.8, 149.8, 155.2, 156.4, 157.9, 162.2 (aromatic carbons + pyrimidine C-5a, C-7, C-8 + thiazole C-4a, C-10a), 171.1 (C=S), 175.0 (C=O) and 181.5 (C=S). IR (KBr) ν: 3310, 3200 (NH), 1718 (C=O), 1605, 1500 cm⁻¹ (aromatic C=C). MS (70 eV): (M⁺) *m/z* 446 (4.0%). Anal. Calcd. for C₁₉H₁₈N₄O₃S₃ (446.57): C(51.10%), H(4.06%), N(12.55%), S(21.54); Found: C(51.0%), H(4.2%), N(12.5%), S(21.8%).

Ethyl 9-(2-hydroxyphenyl)-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1H-thiazolo[3,2-a:4,5-d']dipyrimidine-8-carboxylate (5c) was crystallized from dioxane as pale green crystals, m.p. 295 °C. ¹H-NMR: δ (ppm) 1.28 (t, 3H, CH₃, *J* = 4 Hz), 2.25 (s, 3H, CH₃), 4.16 (q, 2H, CH₂, *J* = 4 Hz), 6.11 (s, 1H, pyrimidine H-9), 6.80–7.20 (m, 4H, Ar-H), 8.22 (s, 1H, OH, D₂O exchangeable), 11.40 (s, 1H, NH, D₂O exchangeable) and 12.25 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR: δ (ppm) 14.3 (CH₃), 17.1 (CH₃), 58.8 (pyrimidine C-5), 67.1 (CH₂), 107.3 (CN), 107.9, 112.4, 120.1, 127.4, 133.3, 138.1, 154.0, 158.1, 158.9, 159.4, 161.0 (aromatic carbons + pyrimidine C-5a, C-7, C-8 + thiazole C-4a, C-10a), 171.4 (C=S), 174.8 (C=O) and 180.7 (C=S). IR (KBr) ν: 3300, 3230 (NH₂), 1710 (C=O), 1600, 1500 cm⁻¹ (aromatic C=C). MS (70 eV): (M⁺) *m/z* 432 (5.0%). Anal. Calcd. for C₁₈H₁₆N₄O₃S₃ (432.54): C(49.98%), H(3.73%), N(12.95%), S(22.24); Found: C(49.8%), H(3.8%), N(13.1%), S(22.1%).

Ethyl 9-(furan-2-yl)-7-methyl-2,4-dithioxo-2,3,4,9-tetrahydro-1H-thiazolo[3,2-a:4,5-d']dipyrimidine-8-carboxylate (5d) was crystallized from dioxane as dark green crystals, m.p. 255 °C. ¹H-NMR: δ (ppm) 1.25 (t, 3H, CH₃, *J* = 4 Hz), 2.15 (s, 3H, CH₃), 4.00 (q, 2H, CH₂, *J* = 4 Hz), 6.11 (s, 1H, pyrimidine H-9), 6.51 (d, 1H, furan-H), 6.78 (m, 1H, furan-H), 7.55 (d, 1H, furan-H), 11.40 (s, 1H, NH, D₂O exchangeable) and 12.25 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR: δ (ppm) 14.5 (CH₃), 17.1 (CH₃), 62.1 (pyrimidine C-9), 66.8 (CH₂), 106.6, 110.0, 123.3, 134.8, 138.7, 142.7, 144.9, 151.4

157.9 (furan carbons + pyrimidine C-5a, C-7, C-8 + thiazole C-4a, C-10a) 171.8 (C=S), 173.9 (C=O) and 181.2 (C=S). IR (KBr) ν : 3310, 3244 (2NH), 2210 (CN), 1722 cm^{-1} (C=O), 1605, 1510 (aromatic C=C). MS (70 eV): (M^+) m/z 406 (4.3 %). Anal. Calcd. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S}_3$ (406.50): C(47.27%), H(3.47%), N(13.78%), S(23.66); Found: C(47.0%), H(3.4%), N(13.8%), S(23.8%).

3.1.4. Ethyl 8-methyl-10-(4-methoxyphenyl)-3-substituted-5-thioxo-2-(un)substituted-10H-thiazolo-[3'',2'':1',2']pyrimido[4',5':4,5]thiazolo[3,2-a]pyrimidine-9-carboxylates **7a–c**

Method A: A solution of **5b** (4.46 g, 0.01 mol) with each of chloroacetone (0.92 g, 0.1 mol), phenacyl bromide (1.99 g, 0.01 mol) or 3-chloropentane-2,4-dione (1.34 g, 0.01 mol) in ethanolic potassium hydroxide solution [prepared by dissolving KOH (0.56 g, 0.01 mol) in ethanol (50 mL)] was heated under reflux for 3 h. A precipitate started to form on hot after 1 h. After cooling, the produced precipitate was filtered off, dried and crystallized from dimethyl formamide.

Method B: The same reactants of method A were heated at 140 °C in microwave oven for 5 min. The reaction mixture was treated in a similar manner to method A to obtain compounds **7a–c**.

Ethyl 3,8-dimethyl-10-(4-methoxyphenyl)-5-thioxo-10H-thiazolo[3'',2'':1',2']pyrimido[4',5':4,5]-thiazolo[3,2-a]pyrimidine-9-carboxylate (7a) was obtained as yellow crystals, m.p. 314 °C. $^1\text{H-NMR}$: δ (ppm) 1.34 (t, 3H, CH_3 , $J = 4$ Hz), 2.45 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.53 (q, 2H, CH_2 , $J = 4$ Hz), 5.77 (s, 1H, pyrimidine H-10), 6.94 (s, 1H, thiazole H-2), 7.15 (d, 2H, Ar-H, $J = 6$ Hz), 7.68 (d, 2H, Ar-H, $J = 6$ Hz). $^{13}\text{C-NMR}$: δ (ppm) 14.7 (CH_3), 19.6 (CH_3), 23.3 (CH_3), 58.2 (OCH_3), 63.1 (CH_2), 67.5 (pyrimidine C-10), 101.6, 106.4, 112.3, 124.1, 128.0, 135.8, 151.3, 155.0, 157.4, 158.3, 158.5, 160.1 (aromatic carbons + pyrimidine C-6a, C-7a, C-8, C-9, C-11a, C-12a + thiazole C-2, C-3), 171.0 (C=O) and 182.5 (C=S). IR (KBr) ν : 1715 (C=O), 1600, 1508 cm^{-1} (Aromatic C=C). MS (70 eV): (M^+) m/z 484 (3.6%). Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_3\text{S}_3$ (484.57): C(54.52%), H(4.16%), N(11.56%), S(19.85); Found: C(54.34%), H(4.2%), N(11.8%), S(19.8%).

Ethyl 8-methyl-10-(4-methoxyphenyl)-3-phenyl-5-thioxo-10H-thiazolo[3'',2'':1',2']pyrimido-[4',5':4,5]-thiazolo[3,2-a]pyrimidine-9-carboxylate (7b) was obtained as orange crystals, m.p. 335 °C. $^1\text{H-NMR}$: δ (ppm) 1.41 (t, 3H, CH_3 , $J = 4$ Hz), 3.17 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.50 (q, 2H, CH_2 , $J = 4$ Hz), 5.75 (s, 1H, pyrimidine H-10), 7.04 (s, 1H, thiazole H-2), 7.22 (d, 2H, Ar-H, $J = 6$ Hz), 7.41 (d, 2H, Ar-H, $J = 6$ Hz), 7.52–7.81 (m, 5H, Ar-H). $^{13}\text{C-NMR}$: δ (ppm) 14.6 (CH_3), 24.7 (CH_3), 58.0 (OCH_3), 63.4 (CH_2), 67.2 (pyrimidine C-10), 103.2, 106.9, 112.8, 115.6, 124.9, 126.2, 128.0, 128.9, 129.3, 135.8, 152.2, 155.1, 157.2, 158.0, 160.4 (aromatic carbons + pyrimidine C-6a, C-7a, C-8, C-9, C-11a, C-12a + thiazole C-2, C-3), 170.7 (C=O) and 183.0 (C=S). IR (KBr) ν : 1718 (C=O), 1600, 1504 cm^{-1} (aromatic C=C). MS (70 eV): (M^+) m/z 546 (2.3%). Anal. Calcd. for $\text{C}_{27}\text{H}_{22}\text{N}_4\text{O}_3\text{S}_3$ (546.68): C(59.32%), H(4.06%), N(10.25%), S(17.60); Found: C(59.2%), H(4.2%), N(10.6%), S(17.8%).

Ethyl 2-acetyl-3,8-dimethyl-10-(4-methoxyphenyl)-5-thioxo-10H-thiazolo[3'',2'':1',2']pyrimido-[4',5':4,5]thiazolo[3,2-a]pyrimidine-9-carboxylate (7c) was obtained as yellow crystals, m.p. 308 °C. $^1\text{H-NMR}$: δ (ppm) 1.35 (t, 3H, CH_3 , $J = 4$ Hz), 2.47 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.50 (q, 2H, CH_2 , $J = 4$ Hz), 5.68 (s, 1H, pyrimidine H-10), 7.00 (d, 2H, Ar-H,

$J = 6$ Hz), 7.55 (d, 2H, Ar-H, $J = 6$ Hz). ^{13}C -NMR: δ (ppm) 12.3 (CH_3), 14.5 (CH_3), 20.8 (CH_3), 25.3 (CH_3), 58.0 (OCH_3), 63.1 (CH_2), 65.5 (pyrimidine C-10), 105.6, 106.2, 112.6, 124.3, 127.2, 135.6, 151.0, 154.3, 155.6, 157.2, 158.1, 160.4 (aromatic carbons + pyrimidine C-6a, C-7a, C-8, C-9, C-11a, C-12a + thiazole C-2, C-3), 170.4 ($\text{C}=\text{O}$), 175.3 ($\text{C}=\text{O}$) and 182.5 ($\text{C}=\text{S}$). IR (KBr) ν : 1715, 1695 (2 $\text{C}=\text{O}$), 1600, 1508 cm^{-1} (aromatic $\text{C}=\text{C}$). MS (70 eV): (M^+) m/z 526 (5.5%). Anal. Calcd. for $\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_3$ (526.08): C(54.73%), H(4.21%), N(10.64%), S(18.27); Found: C(54.5%), H(4.3%), N(10.7%), S(18.1%).

3.2. Antioxidant Screening

3.2.1. Assay for Erythrocyte Hemolysis

Blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat and washed three times with 10 volumes of 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged at 2,500 rpm for 10 min to obtain a constantly packed cell preparation. Erythrocyte hemolysis was mediated by peroxy radicals in this assay system [26]. A 10% suspension of erythrocytes in pH 7.4 phosphate-buffered saline (PBS) was added to the same volume of 200 mM 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37 °C for 24 h. The reaction mixture was then removed, diluted with eight volumes of PBS and centrifuged at 2,500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with eight volumes of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation was measured at 540 nm. The percentage hemolysis was calculated by the equation $(1 - A/B) \times 100\%$. The data were expressed as mean standard deviation. L-Ascorbic was used as a positive control.

3.2.2. Antioxidant Activity Screening Assay—ABTS Method

For each of the investigated compounds 2 mL of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution (60 mM) was added to 3 M MnO_2 solution (25 mg/mL) all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance (A_{control}) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at λ 734 nm. Then, 50 μL of 2 mM solution of the test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation [27]:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested compound.

3.2.3. Bleomycin-Dependent DNA Damage

The assay was done according to Aeschlach *et al.* [28] with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/ mL), bleomycin sulfate (0.05 mg/mL), MgCl₂ (5 mM), FeCl₃ (50 μM) and samples to be tested at different concentrations. L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding 0.5 mL thiobarbituric acid (TBA) (1%, w/v) and 0.5 mL HCl (25%, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.

3.3. Antimicrobial Screening

The newly synthesized heterocyclic compounds were tested for their antimicrobial activity against the following microorganisms: (a) Gram-negative: *Escherichia coli* and *Pseudomonas putide*; (b) Gram-positive: *Bacillus subtilis* and *Streptococcus lactis*; (c) Fungi: *Aspergillus niger* and *Penicillium sp.*; (d) Yeast: *Candida albicans*.

Media: Three types of specific media were used in this study:

Medium (1): For bacteria (Nutrient Medium), consisting of (g/L distilled water): peptone, 5 and meat extract, 3. pH was adjusted to 7.0.

Medium (2): For fungi (Potato Dextrose Medium), consisting of (g/L distilled water): Infusion from potatoes, 4 and D(+)glucose, 20. pH was adjusted to 5.5.

Medium (3): For yeast (Universal Medium), consisting of (g/L distilled water): yeast extract, 3; malt extract, 3; peptone, 5 and glucose, 10. pH was adjusted to 5.5.

For solid media, 2% agar was added. All media were sterilized at 121 °C for 20 min.

Procedure (Filter Paper Diffusion Method) [29]

Proper concentrations of microbial suspensions were prepared from 1 (for bacteria to 3 (for yeast and fungi)-day-old liquid stock cultures incubated on a rotary shaker (100 rpm). In the case of fungi, 5 sterile glass beads were added to each culture flask. The mycelia were then subdivided by mechanical stirring at speed No. 1 for 30 min. Turbidity of microorganisms was adjusted with a spectrophotometer at 350 nm to give an optical density of 1.0. Appropriate agar plates were aseptically surface inoculated uniformly by a standard volume (ca. 1 mL) of the microbial broth culture of the tested microorganism, namely *E. coli*, *P. putide*, *B. subtilis*, *S. Lactis*, *A. Niger*, *Penicillium sp.* and *C. albicans*.

Whatman No. 3 filter paper discs of 10 mm diameter were sterilized by autoclaving for 15 min at 121 °C. Test compounds were dissolved in 80% ethyl alcohol to give final concentration of 5 μg/mL. The sterile discs were impregnated with the test compounds (5 μg/disc). After the impregnated discs have been air dried, they were placed on the agar surface previously seeded with the organism to be tested. Discs were gently pressed with forceps to insure thorough contact with the media. Three discs were arranged per dish, suitably spaced apart, *i.e.*, the discs should be separated by a distance that is equal to or slightly greater than the sum of the diameters of inhibition produced by each disc alone. Each test compound was conducted in triplicate. Plates were kept in the refrigerator at 5 °C for 1 h to

permit good diffusion before transferring them to an incubator at 37 °C for 24 h for bacteria and at 30 °C for 72 h for yeast and fungi.

4. Conclusions

New thiazolopyrimidines have been synthesized using both conventional methods and microwave assisted conditions. The latter methods proved very efficient in reducing reaction times as well as increasing the overall yield of the reactions. The newly synthesized compounds were tested for their antioxidant and antimicrobial activities. Some compounds showed good or moderate antioxidant activity, whereas other compounds showed weak antimicrobial activity.

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Sample Availability: Samples of the compounds are available from the authors.

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