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Research Article

Ecofriendly Synthesis of Bioactive 2-thiobarbituric Acid Derivatives

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Abstract: In this study, five derivatives of 2-thiobarbituric acid were prepared under microwave irradiation (MWI) and conventional heating method. It was found that the preparation time wasreduced from 24 hours to 5-10 minutes by using microwave irradiation method. In microwave irradiation, the yield also comparatively very high (97.50-98.32%) than conventional method (74-78%). FT-IR, ^{1}H-NMR spectral data were used to determine the structures of the compounds. The antimicrobial activity of the synthesized compounds were investigated by using *Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Pseudomonas aeruginosa* bacteia. The zone of inhibition of the compounds were found in the range of 8 to 14 mm. In cytotoxic analysis, the mortality 74-89% were appeared when sample concentration were (0.78-6.25) µg/ml and more than 6.25 µg/ml concentration showed 100% mortality. The antimicrobial and cytotoxic activity of synthesized compoundswas found due to the presence of a reactive and unsaturated ketone.

Keywords: Microwave Irradiation (MWI), 2-thiobarbituric Acid Derivatives, Arylidene Acetophenone, Antimicrobial and Cytotoxic Activity

1. Introduction

In the past two decades, the classic organic chemistry has been rewritten around new approaches that investigate for the perfection of environmentally safer products [1]. Improvement of safe synthetic methodologies for organic reactions is one of the most recent challenges to the organic chemists [2]. The growing concern for the environment demands, the development of eco-friendly and economic process of safe organic synthesis [3-5]. Development of novel synthetic methodologies to facilitate the preparation of desired molecule is a nintense area of research [6, 7]. In this regard, efforts have been made constantly to introduce new methodologies that are efficient and more compatible with the environment [8, 9].

The thiobarbituric acid scaffold consists of a pyrimidine cyclic structure. These compounds have been described as privileged structures, as they provide various points of attachment for a diverse array of structural elements that can be used to target receptor agonists or antagonists owing to the versatile these compounds are more often used for the man kind ailment. Most of the thiobarbiturate derivatives possessed a wide range of biological application in pharmaceutical as well as agrochemicals such as anti-inflammatory, antioxidant, antidepressant, antitumor, antibacterial, sedative, herbicides, fungicidal and antiviral agents etc [10-12].

Molecular modeling is one of important tool that shows exact active site of molecule in pharmacophore [13, 14], therefore, there is a great demand for eco-friendly product which is easily degradable into the nontoxic residue harmless to human being and moreover beneficial to the crop. Led by these considerations the need for novel antimicrobial agents that exhibit broad spectrum and good water solubility has become more pressing.

In the light of the aforementioned facts and the demand for increasingly clean and efficient drug moieties, our interest in the synthesis of biologically active heterocyclic compounds, herein we report the synthesis of 2-thiobarbituric acid derivatives using MWI which is relatively in good yields and to find out the potential biological activities of these compounds.

2. Material and Methods

The classic white ProLine Microwave (720W, 2450MHz) with nine power settings was used for this study. Melting point was was measured with electric-melting point apparatus. In this study, three aromatic aldehydes (benzaldehyde, 4-hydroxybenzaldehyde and 4-chlorobenzaldehyde), two acetophenones (acetophenone and 4-chloroacetophenone) and 2-thiobarbituric acid was used. 3M NaOH, 95% ethanol, rectified sprit and water were used as solvents. All chemicals were used of commercial grade (Mark, Germany) without further purification.

The product was characterized by FT-IR spectrum (KBr) on a Fourier transform spectrometer (FTIR-8300) and by ^{1}H-NMR spectra at room temperature using chloroform-d ($CDCl_3$) with a JEOL EX 270 spectrophotometer at 270MHz.

The rate enhancement for comparable microwave and conventionally heated reactions was calculated by using identical concentration of the following manner:

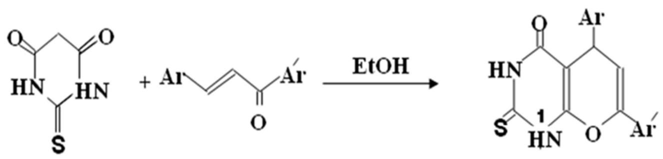
Rate enhancement = (conventional reaction time/microwave reaction time)

Where, for the reactions the conventional reaction time and microwave reaction time were taken to the same extent of completion. In the present work, the reactions were carried out by following a general procedure [15-17].

2.1. Synthesis of 2-thiobarbituric Acid Derivatives (2a-2e)

A mixture of arylidene acetophenone (1a-1e) (0.005mol) and 2-thiobarbituric acid (0.005mol) were dissolved in rectified spirit (25 ml) and water (25 ml) in a 250 ml round-bottomed flask. The flask was equipped with a refluxing condenser placed in a paraffin oil bath on a magnetic stirrer. The reaction mixture was refluxed for 18 hours and the course of the reaction was followed by TLC on silica gel plates (eluting solvent, Pet. ether: EtOAc; 5:1). The mixture was allowed to cool and the solid separated out was dried in air and recrystallized from hot rectified spirit.

In a 250 ml conical flask an equimolar mixture of 2-thiobarbituric acid (2) (0.005 mol) and arylideneacetphenone (1a-1e) (0.005 mol) were dissolved in 25 ml rectified spirit and 25 ml water. The mixture was irradiated with microwave at different power level for several minutes and the progress of the reaction was followed by TLC on silica gel plate (eluting solvent, Pet. Ether: EtOAc; 5:1). The reaction mixture was cooled and the solid was separated out by filtration and recrystallized from hot rectified spirit. The purity of the product was checked by TLC.



	Ar	Ar/
2a	C_6H_5	C_6H_5
2b	C_6H_4OH	C_6H_5
2c	C_6H_5	C_6H_4Cl
2d	C_6H_4OH	C_6H_4Cl
2e	C_6H_4Cl	C_6H_5

Figure 1. *Synthesis of 2-thiobarbituric acid derivatives (2a-2e).*

2.1.1. 5, 7-diphenyl-1, 2, 3, 4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2, 3-d]pyrimidine (2a)

Powder solid, color: whitish; melting point: 291-293°C; IR, *v*:3155, 1700, 1600, 1593, 1446, 1334, 1149, 1110, 1040, 820, 746, 700 (KBr) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ: 11.00 (m, 2H, NH), 7.83-7.24 (m, 10H, Ar-H), 5.97 (d, 1H, 6-H), 4.43 (d, 1H, 5-H).

2.1.2. 5-(4-hydroxyphenyl)-7-phenyl-1, 2, 3, 4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2, 3-d] pyrimidine (2b)

Powder solid, color: whitish; melting point: 189-190°C; IR *v*:3600, 3155, 3030, 1710, 1618, 1593, 1446, 1334, 1145, 1091, 777, 748 (KBr) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ: 10.56 (m, 2H, NH), 7.71-6.93 (m, 9H, Ar-H), 5.66 (d, 1H, 6-H), 4.42 (d, 1H, 5-H), 4.85 (s, 1H, Ar-OH).

2.1.3. 5-phenyl-7-(4-chlorophenyl)-1, 2, 3, 4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2, 3-d] pyrimidine (2c)

Powder solid, color: whitish; melting point: 283-285°C; IR *v*: 3155, 1700, 1645, 1446, 1402, 1311, 1145, 1091, 1035, 825, 790, 748, 617 (KBr) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ: 11.06 (m, 2H, NH), 7.64-6.93 (m, 9H, Ar-H), 5.63 (d, 1H, 6-H), 4.41 (d, 1H, 5-H).

2.1.4. 5-(4-hydroxyphenyl)-7-(4-chlorophenyl)-1, 2, 3, 4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2, 3-d] pyrimidine (2d)

Powder solid, color: whitish; melting point: 274-275°C; IR *v*: 3700, 3155, 1700, 1633, 1446, 1421, 1305, 1178, 1097, 1033, 823, 786, 744, 684 (KBr) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ: 10.96 (m, 2H, NH), 7.56-6.93 (m, 8H, Ar-H), 5.64 (d, 1H, 6-H), 4.42 (d, 1H, 5-H), 4.62 (s, 1H, Ar-OH).

2.1.5. 5-(4-chlorophenyl)-7-phenyl-1, 2, 3, 4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2, 3-d] pyrimidine (2e)

Powder solid, color: whitish; melting point: 282-284°C; IR *v*: 3155, 1700, 1620, 1589, 1446, 1402, 1317, 1163, 1109, 1035, 827, 775, 752, 684 (KBr) cm^{-1}; $^{1}HNMR$ ($CDCI_3$) δ: 11.06 (m, 2H, NH), 7.64-6.93 (m, 9H, Ar-H), 5.68 (d, 1H, 6-H), 4.41 (d, 1H, 5-H).

2.2. Bioassay of Synthesized Compounds

The microorganisms used for the experiment were collected as pure culture from the instituted of Food Science and Technology, BCSIR, Dhaka, Bangladesh. *Aspergillus niger* and *Aspergillus flavus* were taken for the anti-fungal activity test. Cultures of each fungal species were maintainedon potato-dextrose agar (PDA) slants and stored at 4°C and performed by disc diffusion method [18]. On the other hand, the organisms *Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Pseudomonas aeruginosa* were used for anti-bacterial activity test. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria-Bertani (LB) broth medium at 37°C for 24 hours. Cultures of each bacterial strain were maintained on LB agar medium at 4°C [19].

The antimicrobial activity was performed as the methods described previously [20]. Three types of discs were used for anti-bacterial and anti-fungal screening. Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Then discs were soaked with solutions of test samples and dried. Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known anti-bacterial and anti-fungal agent with that of produced by the test sample. In this investigation, kanamycin (30µg/disc) and ketoconazole (30µg/disc) were used as standard reference disc for anti-bacterial and anti-fungal test, respectively. Blank discs were used as negative control which ensures that the residual solvents (left over the discs even after air-drying) and the filter papers were not active themselves. The plates were then inverted and kept in an incubator at 37°C for 24 hours for bacteria and at 28 ±2°C for 48 hours for fungi. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

The cytotoxic activity was performed as described previously [21]. The test samples were dissolved in dimethyl sulfoxide (DMSO) and serial dilution were made as 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781µg/ml. Then each of these test solutions was added to test tubes containing 10 shrimps in simulated brine water (5 ml) and incubated at room temperature for 24 hours. After 24 hours, the mortality percentagesof the shrimps were calculated.

3. Results and Discussion

The final products were obtained by the condensation of 2-thiobarbituric acid with the primary product (1a-1e) under conventional heating and were completed in 18 hours with moderate yield, whereas the same reactions gave excellent yield within few minutes under MWI method. The structural of the compounds was determined by using spectroscopic data. The FT- IR data of the compounds 2a-2e showed broad and sharp bands in the range (ν_{max}) 3155-3100 cm^{-1}indicating the presence of N-H group. The absorption bands at 1710-1680cm^{-1} indicating the presence of C=O group. The bands at 1620-1505 cm^{-1} were assigned to C=C of aromatic rings and C=N of the conjugated form of 2-thiobarbituric acid part. 1460-1400cm^{-1} were indicated to C-C stretching. The bands at 3700-3500 cm^{-1} showing the presence of Ar-OH group and 800-600 cm^{-1} were assigned to aromatic C-Cl group.

The ^{1}H-NMR spectrum of the synthesized compounds showed the N-H protons were strongly deshielded at δ 11.06-10.56 (d). The proton at position 6 appeared as δ 5.97-5.63 (d). the 5-H proton appeared as δ 4.43-4.41 (d). Ar-H group at δ 7.83-6.93 (m) and Ar-OH group at δ 4.85-4.62 (s). All the FT-IR, $^{1}HNMR$ signals are identical to the known compound 2-thiobarbituric acid derivatives [22, 23].

The comparative results of percentage yields and total reaction time for all synthesised compounds by both conventional method and microwave-assisted method was summarized in Table 1. It was found that there is remarkable improvement in percentage yields and also drastic reduction in total reaction time by using microwave irradiation. This would be highly advantageous for drug discovery in laboratories where small amounts of different analogues have to be synthesised in short periods of time. Microwave-assisted synthesis is quicker, high yielding, environment friendly and shows cleaner chemistry.

Table 1. *Comparative study for the synthesis of 2-thoibarbituric acid derivatives.*

Compounds	Conventional method		Microwave method		
	Time (hr)	Yield (%)	Time (min)	Power (W)	Yield (%)
2a	18	74.00	8	320	98.00
2b	18	72.00	8	320	97.50
2c	18	73.00	8	320	98.00
2d	18	74.00	8	320	95.00
2e	18	78.00	8	160	98.32

The synthesised barbituric acid derivatives were screened for their antibacterial activity against both Gram positive and Gram negative organisms by disc diffusion method using Kanamycin and Ketoconazole as the standard and methanol as the vehicle. *Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Pseudomonas aeruginosa* are used as the organisms. All the compounds showed resistivity against *Staphylococcus aureus* and *Bacillus megaterium*. The diameters of zone of inhibition were 8-14 mm. However. The two Gram negative organism namely *Escherichia coli* and*Pseudomonas aeruginosa* were showed zone of inhibition 8-11 mm to most of the compounds tested (Table 2).

Table 2. *Antimicrobial activities of the synthesized compounds.*

Tested Sample	Name of Bacteria				Name of Fungi	
	S. aureus	*B. megaterium*	*P. aeruginosa*	*E. coli*	*A. niger*	*A. flavus*
	Diameter of Zone of Inhibition (mm)					
2a	9	8	-	-	23	23
2b	13	14	11	10	23	24
2c	11	12	9	-	14	26
2d	12	10	8	8	14	17
2e	12	10	8	8	14	17
Ketoconazole	-	-	-	-	22	26
Kanamycin	28	29	28	27	-	-

Antifungal activity of all the synthesized compounds were also screened against *Aspergillus niger and Aspergillus flavus* by disc diffusion method using ketoconazole (30 µg/disc) as the standard. As shown in Table 2 both the fungal strains were found to be moderately sensitive to all the tested compounds with zone of inhibition 14-24 mm.

The cytotoxic activities of the synthesized compounds were determined by using brine shrimp lethality bioassay. The mortality percentages for all the tested samples were found to be very high. The mortality percentage of the tested compounds has shown in Table 3. Sample concentration 0.78-6.25 (µg/ml) showed the mortality of 74-89%, whereas 12.5-50 (µg/ml) concentration showed 100% mortality. From this study, it is evident that all the test samples were lethal to brine shrimp nauplii. These positive results suggested that they may contain antitumor or pesticidal activity.

Table 3. *Cytotoxic activities of the synthesized compounds.*

Tested Sample	Sample Concentration (µg/ml)						
	0.78	1.56	3.125	6.25	12.5	25	50
	Mortality (%)						
2a	74	74	89	89	100	100	100
2b	49	78	89	89	100	100	100
2c	86	86	89	89	100	100	100
2d	57	79	89	89	100	100	100
2e	86	86	100	100	100	100	100

4. Conclusion

In this study, the synthesis procedure offers reduction in reaction time, operation simplicity, excellent yields without undesirable side products, cleaner reaction and easy work-up in Microwave-assisted syntheses. These synthesis process also produce improved yield as compared to the conventional heating with reaction time reduced from hours to minutes. Microwave-assisted syntheses method also called eco-friendly process because it needs low amount of chemicals for making the compounds. In other words, as a modest work of green chemistry, it is a viable and feasible method for performing the synthesis of drug intermediates and chemicals.

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