



Green Chemistry Approach for Synthesis of Bioactive 2-Thiobarbituric Acid Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAS designed the study and wrote the protocol, author KK managed the analyses of the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors KN and JA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The following study is aimed to prepare five derivatives of 2-thiobarbituric acid under microwave irradiation (MWI) and conventional heating method and investigate their biological activities.

Study Design: A classic white ProLine Microwave (720 W, 2450 MHz) with nine power settings was used for this study.

Place and Duration of Study: Department of Applied Chemistry & Chemical Engineering, Islamic University, Kushtia, Bangladesh, between June 2012 and February 2014.

Methodology: Five derivatives of 2-thiobarbituric acid were synthesised by using Microwave (720 W, 2450 MHz) with nine power settings and the conventional method. The compounds were investigated by using *Staphylococcus aureus*, *Bacillus megaterium*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. The cytotoxic activity was performed in the test tubes containing ten shrimps in simulated brine water.

Results: It was found that the preparation time was reduced from 24 hours to 5-10 minutes by using microwave irradiation method. In microwave irradiation, the yield also comparatively very high

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(97.68-98.50%) than conventional method (76-80%). The antimicrobial activity of the synthesised compounds was investigated by measuring the zone of inhibition of the compounds. In the cytotoxic analysis, the mortality 78-89% appeared when sample concentration was (0.78-6.25) µg/ml and more than 6.25 µg/ml concentration showed 100% mortality.

Conclusion: Microwave-assisted synthesis of an improved method that yields to compare some of the conventional heating with reaction time reduced from hours to minutes. The compounds also showed potential antimicrobial and cytotoxic activity of the following MWI prepared derivatives.

Keywords: Microwave irradiation (MWI); 2-thiobarbituric acid derivatives; arylidene acetophenone; antimicrobial and cytotoxic activity.

1. INTRODUCTION

In recent years it has recognized that the science of chemistry is central to addressing the problems facing the environment. Through the utilization of the various subdisciplines of chemistry and the molecular sciences, there is an increasing appreciation that the emerging area of green chemistry is needed in the design and attainment of sustainable development. A central driving force in this increasing awareness is that green chemistry accomplishes both economic and environmental goals simultaneously. Through the use of sound, fundamental scientific principles [1]. Development of novel synthetic methodologies to facilitate the preparation of the desired molecule is an intense area of research [2,3]. In this regard, efforts have been made constantly to introduce new methodologies that are efficient and more compatible with the environment [4,5]. Investigations have shown that microwave irradiation is considered to be green, more environments friendly, efficient, faster, stable and cleaner than the conventional heating methods [6].

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, anti-tumor, antibacterial, sedative, herbicides, fungicidal and antiviral agents etc. [7,8].

Molecular modelling is one of an important tool that shows the exact active site of the molecule in pharmacophore [9]. Therefore,

there is a great demand for an eco-friendly product which is easily degradable into the nontoxic residue harmless to a 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 facts above 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. MATERIALS AND METHODS

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

The product was characterised by FT-IR spectrum (KBr) on a Fourier transform spectrometer (FTIR-8300) and by ¹H-NMR spectra at room temperature using chloroform-d (CDCl₃) with a JEOL EX 270 spectrophotometer at 270 MHz.

The rate enhancement for comparable microwave and conventionally heated reactions were calculated by using same concentration in the following manner:

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

Where the reaction time in the conventional method and microwave irradiation method were different. In the present work, the reactions were carried out according to Ahmed et al. [10].

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

A mixture of arylidene acetophenone (1a-1e) (0.005 mol) and 2-thiobarbituric acid (0.005 mol) was dissolved in aqueous ethanol as a solvent in a 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, then followed by condensation reaction mechanism various derivatives were formed. The course of the reaction was confirmed 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 arylideneacetophenone (1a-1e) (0.005 mol) were dissolved in aqueous ethanol as solvent. The mixture was irradiated with microwave at a 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. TLC checked the purity of the product.

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

Powder solid, color: whitish; melting point: 285-287°C; IR, ν : 3600, 3155, 1710, 1618, 1523, 1446, 1091, 745, 680 (KBr) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ : 10.56 (m, 2H, NH), 7.68-7.07 (m, 9H, Ar-H), 5.95(d, 1H, 6-H), 4.42 (d, 1H, 5-H), 4.82 (s, 1H, Ar-OH).

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

Powder solid, color: whitish; melting point: 246-248°C; IR ν : 3155, 3010, 1700, 1620, 1501, 1446, 1404, 1317, 1089, 1033, 825, 777. (KBr) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ : 10.42 (m, 2H, NH), 7.72-7.29 (m, 8H, Ar-H), 5.68(d, 1H, 6-H), 4.41 (d, 1H, 5-H).

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

Powder solid, color: whitish; melting point: 275-277°C; IR ν : 3700, 3155, 1710, 1620, 1512, 1435, 1145, 1100, 775 (KBr) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ : 10.42 (m, 2H, NH), 7.53-7.25 (m, 8H, Ar-H), 5.93 (d, 1H, 6-H), 4.45 (d, 1H, 5-H), 4.72 (s, 1H, Ar-OH).

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

Powder solid, color: whitish; melting point: 260-262°C; IR ν : 3155, 1710, 1595, 1510, 1444, 1261, 1111, 654. (KBr) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ : 10.96 (m, 2H, NH), 7.79-6.71 (m, 9H, Ar-H), 5.81 (d, 1H, 6-H), 4.33 (d, 1H, 5-H), 3.83 (s, 1H, Ar- CH_3O).

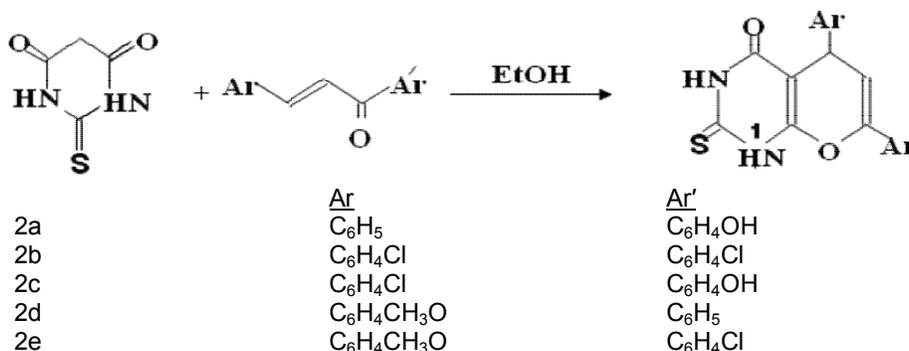


Fig. 1. Synthesis of 2-thiobarbituric acid derivatives (2a-2e)

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

Powder solid, color: whitish; melting point: 254-256°C; IR ν : 3150, 1700, 1620, 1505, 1444, 1423, 1254, 1087, 740, (KBr) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ : 10.63 (m, 2H, NH), 7.56-6.98 (m, 8H, Ar-H), 5.74 (d, 1H, 6-H), 4.27 (d, 1H, 5-H), 3.48 (s, 1H, Ar- CH_3O).

2.2 Bioassay of Synthesized Compounds

The microorganisms used for the experiment were collected as a 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 maintained on potato-dextrose agar (PDA) slants and stored at 4°C and performed by disc diffusion method [11]. 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 [12].

The antimicrobial activity was performed as the methods described previously [13]. Three types of discs were used for anti-bacterial and anti-fungal screening. A measured amount of each test sample was dissolved in a 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 the 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 (30 $\mu\text{g}/\text{disc}$) and ketoconazole (30 $\mu\text{g}/\text{disc}$) were used as standard reference disc for the anti-bacterial and anti-fungal test, respectively. Blank discs were used as a 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 $28 \pm 2^\circ\text{C}$ 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 [14]. The test samples were dissolved in dimethyl sulfoxide (DMSO) and serial dilution was made as 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 $\mu\text{g}/\text{ml}$. 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 percentages of 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 a 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 1710-1680 cm^{-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 the 2-thiobarbituric acid part. 1460-1400 cm^{-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 an aromatic C-Cl group and 1240-1265 cm^{-1} indicates Ar- CH_3O group.

The $^1\text{H-NMR}$ spectrum of the synthesized compounds showed the N-H protons were strongly deshielded at δ 10.96-10.56 (d). The proton at position 6 appeared as δ 5.95-5.68 (d), the 5-H proton appeared as δ 4.45-4.27 (d). Ar-H group at δ 7.79-6.71 (m) and Ar-OH group at δ 4.82-4.72 (s). All the FT-IR, $^1\text{H-NMR}$ signals are identical to the known compound 2-thiobarbituric acid derivatives [15,16].

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 a remarkable improvement in percentage yields and also a

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

Compounds	Conventional method		Microwave method		
	Time (hr)	Yield (%)	Time (min)	Power (W)	Yield (%)
2a	18	78.00	8	320	98.00
2b	18	76.00	8	80	97.68
2c	18	80.00	8	80	98.00
2d	18	78.00	8	80	98.27
2e	18	80.00	8	80	98.50

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 [17,18].

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 as the standard and methanol as the vehicle. The obtained antimicrobial activity of tested compounds could be correlated to structural variations and modifications of the respective compounds. On the basis of structure-activity relationship (SAR) studies, the results suggested that the antimicrobial activity of 2-Thiobarbituric Acid derivatives was markedly influenced by various substituents on the benzene ring and the incorporation of the electron withdrawing group caused enhancement in activity against most test microorganisms. 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 a zone of inhibition 6-10 mm to most of the compounds tested (Table 2).

Antifungal activity of all the synthesized compounds was 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 a 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 2. Antimicrobial activities of the synthesized compounds

Tested sample	Name of bacteria				Name of fungi	
	<i>S. aureus</i>	<i>B. megaterium</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>A. niger</i>	<i>A. flavus</i>
The diameter of zone of inhibition (mm)						
2a	14	11	10	8	16	17
2b	12	10	6	9	14	16
2c	12	12	9	7	19	20
2d	12	11	10	10	16	16
2e	11	8	6	-	18	16
Ketoconazole	-	-	-	-	22	26
Kanamycin	28	29	28	27	-	-

Table 3. Cytotoxic activities of the synthesized compounds

Tested sample	Sample concentration ($\mu\text{g/ml}$)						
	0.78	1.56	3.125	6.25	12.5	25	50
	Mortality (%)						
2a	49	78	89	89	100	100	100
2b	89	89	100	100	100	100	100
2c	57	79	89	89	100	100	100
2d	68	68	89	89	100	100	100
2e	84	87	100	100	100	100	100

4. CONCLUSION

In this study, the synthesis procedure offers a 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 a 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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