

## Antibacterial, Antiurease, and Antioxidant Activities of Some Arylidene Barbiturates

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**Abstract** Some series of arylidene barbiturates and thiobarbiturates were evaluated for their antibacterial, antioxidant, and urease inhibition activities. The arylidene barbiturates and thiobarbiturates were tested for antimicrobial activity using the agar well diffusion technique against 13 bacteria. The synthesized compounds (1a–g) were screened for antiurease and antioxidant activities. The results showed that the synthesized compounds (1a–g) had effective antiurease, antioxidant, and antibacterial activities.

**Keywords** Arylidene barbiturates · Antibacterial · Antioxidant · Urease inhibition activity

### Introduction

Barbituric acids have attracted the attention of the pharmaceutical scientist for over 100 years due to their therapeutic value [1]. Barbituric acid and its derivatives have exhibited biological activities such as in antibacterial, hypotensive, and antisclerotics [2], sedative, hypnotic, antispasmodic, anticonvulsant, and local anesthetic drugs [3, 4] as well as in anticancer [5], anti-inflammatory [6], and matrix metalloproteinase inhibitors [7].

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In recent years, barbiturate and thiobarbiturate derivatives very much attracted considerable attention owing to their various biological effects such as inhibiting collagenase-3 [8] matrix metalloproteinases [9], recombinant cytochrome P<sub>450</sub> enzymes [10], methionine aminopeptidase-1 [11], tyrosinase [12], and urease [13]. Also, antioxidant activity values of some arylidene barbiturates have been studied [14].

Arylidene barbiturates are important members of the pyrimidine family. The major importance of these compounds has been centered on their application as useful precursors in the preparation of new heterocyclic bioactive molecules [15] and as potential selective oxidizing agents [16] and for the unsymmetrical synthesis of disulphide [17]. Some of them have been studied as nonlinear optical materials [18]. Due to their importance in the above-mentioned, very different methods for barbituric acid synthesis were reported in the literature. Arylidene barbiturates may be synthesized by Knoevenagel condensation reaction of barbituric/thiobarbituric acid with various aldehydes [19–21]. The isolated products are mono-substituted as well as di-substituted [22]. To achieve the formation of only one mono-substituted condensation product, various catalysts has been investigated [1, 21, 23].

Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, stroke, inflammation, diabetes mellitus, and cancer [24, 25]. Reactive oxygen species (ROS) include free radicals such as superoxide anion, hydroxyl radical, singlet oxygen, and hydrogen peroxide can cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes [26]. The tissue injury caused by ROS may include DNA damage, protein damage, and important enzymes in human body [27]. These events could consequently lead to the occurrence of various free-radical-related diseases. Antioxidants are extensively studied for their capacity to protect organisms and cells from damage that is induced by oxidative stress. Nowadays, antioxidants arouse researchers' interest in both medical plants and synthetic compounds. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone, have been widely used in the food industry to prevent oxidative deterioration, but BHA and BHT are suspected of being responsible for liver damage and carcinogenesis [28]. Scientists in various disciplines have become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells.

Urease (urea amidohydrolase; EC 3.5.1.5) is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbamate [29]. The resulting carbamate spontaneously decomposes to yield a second molecule of ammonia and carbon dioxide. It is present in a variety of plants, algae, fungi, and bacteria in soil enzymes [30]. High concentrations of ammonia arising from these reactions, as well as the accompanying pH elevation, have important negative implications in medicine and agriculture [31, 32]. Urease is involved in the pathogenesis of hepatic encephalopathy, hepatic coma urolithiasis, pyelonephritis, ammonia, and urinary catheter encrustation [33]. It is also a major cause of pathologies induced by *Helicobacter pylori* as this allows bacteria to survive at the low pH of the stomach and hence plays an important role in producing peptic and gastric ulcers [34]. In the near past, a number of compounds have been proposed as urease inhibitors to reduce environmental problems and enhance the uptake of urea nitrogen by plants [35, 36]. Many urease inhibitors have been investigated in the past decades, such as phosphorodiamidates, hydroxamic acid derivatives, and imidazoles, but most of these compounds are too toxic or unstable to allow their use in vivo. Thus, the search is still on for novel urease inhibitors with promising levels of activity [37].

In this study, we have investigated antioxidant, antimicrobial, and urease inhibition activities of some arylidene barbituric acids (1a–g) derivatives. Urease inhibitors with antioxidant and antimicrobial properties may be proved as hallmark for the development

of new antiulcer drugs which will not only clear the *H. pylori* infection but also the induced oxidative stress be redressed.

## Materials and Methods

### General

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Antioxidant activities of samples were determined in a spectrophotometer (UV-1240, Shimadzu, Japan).

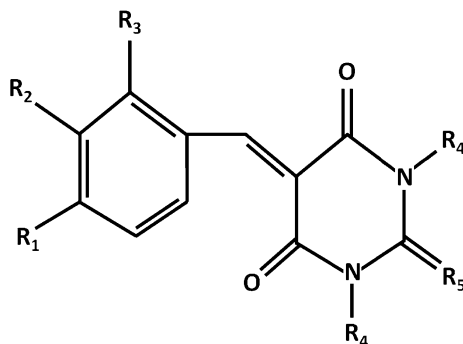
Studied compounds 1a [38], 1b [11], 1c [39], 1d [11], 1e [40], 1f [41], and 1g [42] were prepared by using literature procedures (Scheme 1; Table 1).

### Microbial Strains

The synthesized compounds were tested individually against 13 gram-positive and gram-negative bacteria species. The bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) and were as follows: *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 14028, *Staphylococcus epidermidis* ATCC 12228, *Proteus vulgaris* ATCC 13315, *Yersinia pseudotuberculosis* ATCC 911, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 7644, and *E. faecalis* ATCC 51299. All synthesized compounds were weighed and dissolved in dimethyl sulfoxide (DMSO) to prepare extract stock solutions.

### Antibacterial Activity with the Agar Well Diffusion Method

Antibacterial activity was screened by agar well diffusion method [43, 44]. Test bacteria was cultivated overnight in Mueller Hinton Broth medium at 37°C. Then, Mueller Hinton Agar plates were swabbed (sterile cotton swabs) with 100  $\mu$ L of test bacteria culture which has concentration  $OD_{625}=0.08-0.1$  (approximately  $1 \times 10^7-1 \times 10^8$  CFU/mL). Using the sterile cork borer, the well (6 mm) was made into the each plate. The test substances were prepared in DMSO which showed no zone of inhibition and acts as a negative control. The



**Scheme 1** Studied compounds

**Table 1** Some arylidene barbiturate derivatives used in this study (1a–g)

Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
1a	H	H	OH	H	O
1b	OH	H	H	H	O
1c	H	H	OH	H	S
1d	OH	H	H	H	S
1e	H	H	OH	CH <sub>3</sub>	O
1f	OH	H	OH	CH <sub>3</sub>	O
1g	H	OH	H	CH <sub>3</sub>	O

concentrations of the test substances were prepared as 30 mg/mL. Fifty microliters of the test substances was added into the wells. Simultaneously, an antibiotic, ampicillin (60 mg/mL), was used as positive control and were tested against the microorganisms. Twenty-five microliters of the antibiotic was added into the wells. Then the plates were incubated at 37°C for 16–24 h. After the incubation period, the diameter of the inhibition zones of each well was measured. Each experiment was performed in triplicate.

#### Antioxidant and Urease Enzyme Inhibition Activity

Cuprac-reducing antioxidant capacity of the some arylidene derivatives was determined according to the method described by Apak et al. [45]. Urease inhibitory activity was determined according to Van Slyke and Archibald [46].

## Result and Discussion

The anti-microbial activity of compounds 1a–g and ampicillin was assayed against the gram-positive (G+) (*E. faecalis* ATCC 29212, *S. epidermidis* ATCC 12228, *S. aureus* ATCC 25923, *B. subtilis* ATCC6633, *L. monocytogenes* ATCC 7644, and *E. faecalis* ATCC 51299) and gram-negative (G–) (*E. cloacae* ATCC 13047, *S. typhimurium* ATCC 14028, *P. vulgaris* ATCC 13315, *Y. pseudotuberculosis* ATCC 911, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, and *E. coli* ATCC 35218) microorganisms. Anti-microbial activity of the studied bacteria was qualitatively and quantitatively assessed by evaluating the presence of inhibition zones and minimal inhibition concentration (MIC) values [47–50].

Four compounds (1a, 1e, 1f, 1g) showed anti-microbial activity against G+ and G– bacteria tested, and the results are given in Tables 2 and 3. Four compounds (1a, 1e, 1f, 1g) were found to be same active as ampicillin against almost all tested strains. The maximum inhibition zones and MIC values for the bacterial strains that were sensitive to the studied arylidene barbiturate compounds (1a–g) were in the range of 10–27 mm and from 10.30 to 650 µg/mL, respectively (Table 3).

The results indicate that it is clear that the hydroxyl group of the aryl chain influenced the broadening of the spectrum of anti-microbial activity and MIC values of the investigated compounds. Decreasing of the activity was observed in the case of p–OH group in aryl chain and due to replacing O with S on barbiturate rings. Thioanalogues of arylidene barbiturates have no antibacterial activity as compared with other studied barbiturates. On the other hand, when a hydroxy group was present at o-position in ring-aryl, the activity increased accordingly in arylidene barbiturates. Thus, increase in activity is probably due to the resonance effect of –OH

**Table 2** Antimicrobial screening data for the studied compounds (1a–g)

	Zone of inhibition (mm)							AMP	DMSO
	1a	1b	1c	1d	1e	1f	1g		
<i>E. cloacae</i> ATCC 13047	12	–	–	–	13	14	–	–	–
<i>E. faecalis</i> ATCC 29212	19	–	–	–	12	12	17	26	–
<i>S. typhimurium</i> ATCC 14028	13	–	–	–	–	16	–	19	–
<i>S. epidermidis</i> ATCC 12228	25	–	–	–	18	20	16	<sup>a</sup>	–
<i>P. vulgaris</i> ATCC 13315	26	–	–	–	–	20	–	25	–
<i>Y. pseudotuberculosis</i> ATCC 911	–	–	–	–	18	17	15	<sup>a</sup>	–
<i>S. aureus</i> ATCC 25923	25	–	–	–	20	13	23	35	–
<i>P. aeruginosa</i> ATCC 27853	–	–	–	–	–	–	–	32	–
<i>K. pneumoniae</i> ATCC 13883	–	–	–	–	–	19	–	–	–
<i>B. subtilis</i> ATCC 6633	14	–	–	–	15	16	15	31	–
<i>E. coli</i> ATCC 35218	11	–	–	–	–	19	–	15	–
<i>L. monocytogenes</i> ATCC 7644	27	–	–	–	15	17	19	27	–
<i>E. faecalis</i> ATCC 51299	20	–	–	–	11	10	15	28	–

AMP ampicillin (60 mg/mL) as positive control, DMSO negative control

<sup>a</sup>Not determined

group that is more prominent at o-position than m-position and p-position. Above results show that electron-donating groups like hydroxyl in o-position on the arylchain and oxygen in carbonyl group on the barbiturate rings are responsible for the antibacterial activity of arylidene barbiturates.

The reducing power has been used as one of the antioxidant capability indicators. There was a correlation found between the reducing capabilities and substituents. The reason for the higher

**Table 3** Minimum inhibition concentration values of the studied compounds (1a–g)

	MIC values (μg/mL)						
	1a	1b	1c	1d	1e	1f	1g
<i>E. cloacae</i> ATCC 13047	325.00	–	–	–	325.00	81.25	–
<i>E. faecalis</i> ATCC 29212	162.50	–	–	–	81.25	20.60	81.25
<i>S. typhimurium</i> ATCC 14028	650.00	–	–	–	–	81.25	–
<i>S. epidermidis</i> ATCC 12228	40.12	–	–	–	81.25	40.12	81.25
<i>P. vulgaris</i> ATCC 13315	81.25	–	–	–	–	81.25	–
<i>Y. pseudotuberculosis</i> ATCC 911	–	–	–	–	325.00	162.50	650.00
<i>S. aureus</i> ATCC 25923	162.50	–	–	–	162.50	162.50	162.50
<i>P. aeruginosa</i> ATCC 27853	–	–	–	–	–	–	–
<i>K. pneumoniae</i> ATCC 13883	–	–	–	–	–	40.12	–
<i>B. subtilis</i> ATCC 6633	81.25	–	–	–	81.25	20.60	162.50
<i>E. coli</i> ATCC 35218	325.00	–	–	–	325.00	81.25	325.00
<i>L. monocytogenes</i> ATCC 7644	325.00	–	–	–	162.50	81.25	81.25
<i>E. faecalis</i> ATCC 51299	10.30	–	–	–	81.25	81.25	10.30

reducing power capacity of the compounds can be explained by looking into the structure of compounds. In our study, the barbiturates derivatives showed marked cupric ion ( $\text{Cu}^{+2}$ )-reducing ability. Cupric ion ( $\text{Cu}^{+2}$ )-reducing ability of barbiturate derivatives is shown in Table 4. Cupric ion ( $\text{Cu}^{+2}$ )-reducing capability of barbiturates derivatives by cuprac method was found to be concentration-dependent (25–100  $\mu\text{g}/\text{mL}$ ). Compounds 1b and 1f showed the lowest cupric ion ( $\text{Cu}^{+2}$ )-reducing capability (Table 3). Compounds 1g, 1e, and 1a exhibited the moderate effect on reducing power. The highest reducing capacity was found for 1c and 1d barbiturate derivatives. Thioanalogues of arylidene barbiturates have antioxidant activity as compared with other studied barbiturates. Cupric ion ( $\text{Cu}^{+2}$ )-reducing power of barbiturates

**Table 4** Cupric ions reducing antioxidant capacity of different concentration of barbiturate derivatives

Compounds	Barbiturate derivatives concentration ( $\mu\text{g}/\text{mL}$ )	Absorbance <sup>a</sup>
1a	25	0.253±0.012
	50	0.296±0.017
	75	0.372±0.015
	100	0.408±0.014
1b	25	0.206±0.009
	50	0.252±0.014
	75	0.308±0.003
	100	0.342±0.005
1c	25	0.116±0.003
	50	1.242±0.012
	75	1.330±0.006
	100	1.429±0.044
1d	25	1.035±0.006
	50	1.196±0.018
	75	1.344±0.041
	100	1.372±0.020
1e	25	0.225±0.007
	50	0.294±0.008
	75	0.348±0.005
	100	0.508±0.006
1f	25	0.197±0.022
	50	0.260±0.004
	75	0.279±0.011
	100	0.358±0.006
1g	25	0.293±0.008
	50	0.355±0.004
	75	0.420±0.013
	100	0.647±0.008
BHT	25	0.586±0.016
	50	0.997±0.022
	75	1.394±0.016
	100	1.575±0.009

<sup>a</sup>Mean±SD

derivatives and BHT at the same concentration (100  $\mu\text{g}/\text{mL}$ ) exhibited the following order: BHT>1c>1d>1g>1e>1a>1f>1b.

Certain synthetic compounds have shown potential urease inhibition such as hydroxy-urea, flurofamide, and hydroxyamic acid; however, the *in vivo* use of some of these has been prohibited because of their toxicity or instability; for instance, acetohydroxyamic acid has been demonstrated to be teratogenic in rats [51]. The discovery of potent and safe urease

**Table 5** The urease inhibitory activity of different concentrations of barbiturates derivatives.

Compounds	Barbiturate derivatives concentration( $\mu\text{g}/\text{mL}$ )	Inhibition (%) <sup>a</sup>	IC <sub>50</sub> ( $\mu\text{M}$ ) <sup>a</sup>
1a	0.001	17.83±1.55	2.582±0.114
	0.01	27.67±2.21	
	0.1	45.66±6.00	
	1	63.07±0.96	
1b	0.001	21.90±1.59	1.841±0.081
	0.01	32.93±2.50	
	0.1	49.36±3.17	
	1	72.06±2.02	
1c	0.001	25.36±2.58	0.054±0.006
	0.01	37.61±4.21	
	0.1	61.16±3.52	
	1	73.03±1.08	
1d	0.001	23.61±3.10	0.038±0.003
	0.01	53.44±4.33	
	0.1	62.55±2.50	
	1	75.77±1.57	
1e	0.001	31.40±2.46	0.036±0.002
	0.01	57.86±3.56	
	0.1	67.98±3.94	
	1	77.09±2.52	
1f	0.001	22.88±1.10	0.050±0.004
	0.01	38.29±2.75	
	0.1	52.17±4.10	
	1	73.84±0.83	
1g	0.001	37.61±3.40	0.033±0.151
	0.01	51.86±1.30	
	0.1	63.85±2.09	
	1	76.03±2.33	
Thiourea	0.001	34.92±0.85	8.825±1.601
	0.01	38.72±1.75	
	0.1	43.12±1.48	
	1	45.36±1.04	
Hydroxyurea	0.001	29.76±2.36	7.418±0.012
	0.01	33.24±0.95	
	0.1	36.95±2.13	
	1	38.61±1.43	

<sup>a</sup>Mean±SD

inhibitors has been a very important area of pharmaceutical research due to the involvement of urease in different pathological conditions.

Urease inhibitor activity of was given in Table 5. Table 5 shows that the arylidene barbiturate derivatives are more potent than the standard inhibitor of urease, hydroxyurea, and thiourea. Thiourea and hydroxyurea are the main examples of the substrate-like urease inhibitors. The inhibition was increased with increasing barbiturate concentration.  $IC_{50}$  values for barbiturate compounds were found to be 0.033–2.582  $\mu\text{M}$ . Compounds 1g and 1e proved to be the most potent showing an enzyme inhibition activity with an  $IC_{50}$ =0.033–0.036  $\mu\text{M}$ . The least active compound 1a had an  $IC_{50}$ =2.582  $\mu\text{M}$ . All of the compounds showed high potent activity than that of standard hydroxyurea ( $IC_{50}$ =7.418  $\mu\text{M}$ ) and thiourea ( $IC_{50}$  = 8.825  $\mu\text{M}$ ). In the other study, synthesized polyphenol compounds ( $IC_{50}$ =22–43.8  $\mu\text{M}$ ) had a lower urease inhibitor activity than in our study. The discovery that barbituric acid inhibits urease was made by Gray et al., who observed that certain cyclic urea compounds (i.e., barbituric and thiobarbituric acids) were capable of inhibiting bacterial and jack-bean urease [52, 53]. Tanaka et al. demonstrated that some alpha hydroxyketone derivatives show urease inhibitory activity [54]. Xiao et al. reported some synthesized pyrogallol and catechol derivatives as *H. pylori* urease inhibitors [55]. Many synthetic and natural apple polyphenols have shown inhibitory activity against urease activity [56]. Rauf et al. reported that some barbituric acid- and thiobarbituric acid-derived sulfonamides were urease inhibitors [57]. In our study, the synthesized compounds can be regarded as substrate-like inhibitors based on their structural similarity to the natural substrate of urease, urea, and thiourea. Since all the synthesized barbiturate compounds promise urease inhibitor activity, this may be due to their basic skeleton.

The presence of –S and –O of barbiturate derivatives isomers in this study may together play a great role on urease inhibition activity.

## Conclusions

In our study, some arylidene barbiturates have been synthesized, and their antibacterial, antiurease, and antioxidant activities were evaluated. The results showed that all of arylidene barbiturate derivatives exhibited antiurease and antioxidant activities. According to our results, there is a correlation between antiurease, antibacterial, and antioxidant activities of compounds and substituents. Among the synthesized compounds, compounds 1c and 1d were found to be the most active reducing agent. Compounds 1g, 1e, 1d, and 1f were determined to be have the highest antiurease activity. Also, compounds 1a, 1e, 1f, and 1g were showed to be the highest antibacterial activity. These arylidene barbiturates can be used in agriculture and pharmacy industries due to their excellent antibacterial, antiurease, and antioxidant activities.

**Conflict of interest** The authors declare no financial or other relationship that might lead to a conflict of interest.

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