Antibacterial and Antifungal Activities of 5-Arylidene-*N,N*-Dimethylbarbiturates Derivatives

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Summary: A series of *N,N*-dimethyl-arylidene barbiturates **1-22** has been re-synthesized and evaluated against a number of Gram-positive, Gram-negative bacteria and some fungal strains. Most of the compounds were found to be active against a number of Gram-positive, Gram-negative and also displayed anti-fungal activities.

Keywords: N, N-Dimethylbarbituric acid, antibacterial, antifungal

Introduction

Barbituric acid, a heterocyclic organic compound based on pyrimidine heterocyclic skeleton, is a parent compound of a class of drugs called barbiturates. Though barbituric acid itself is pharmacologically inactive, however, its derivatives showed high hypnotic and sedative actions [1]. Furthermore they are used as hypnotics, sedatives, anticonvulsants and anesthetics [2]. Some of the barbituric acid analogs have been reported to show antimicrobial [3], antifungal [4], antiviral [5], antidiabetic [6], and anti-inflammatory activities [7]. They are broadly used in the treatment of various diseases and this is probably due to the vulnerability of barbiturates to rapid metabolic attacks, their degradation occurs within the body due to the presence of an acidic hydrogen in their skeleton at C-5 position [8]. Large oral intake of barbiturate directly affects the cardiovascular system and induces coma, peripheral vasodilation, increased blood sugar level and low blood pressure [2]. Literature reports revealed that barbiturates have additional pharmacological potential as anti-AIDS agents, anticancer remedies and immunomodulating capabilities [9,10].

Barbituric acid, itself is used as precursor for the syntheses of a wide range of materials such as plastics, textiles [11], pigments [12], and vitamin B2 (riboflavin) [13]. Barbiturates are used as intermediates in the benzyl barbituric derivatives, heterocyclic compounds and oxadeazaflavines [14]. Recently, they have been used as an initiator for free radical polymerization reactions [15] and in nano-science applications. Organic molecules based on barbiturates and thiobarbiturates have been investigated as promising non-linear optical materials and dyes [14,16]. 2-Thiobarbituric acid is use as inhibitor of corrosion phenomena and for monitoring auto-oxidation mechanisms of fats and oils [16].

Previously we have reported *N, N*-dimethylbarbituric acid derivatives as a DPPH radical scavenger and xanthine oxidase inhibitory activity [17,18]. Now we are reporting the antimicrobial activities of compounds **1-22**. We have re-synthesized compounds **1-22**, and subjected for screening against a number of Gram-positive and Gram-negative bacterial and fungal strains.

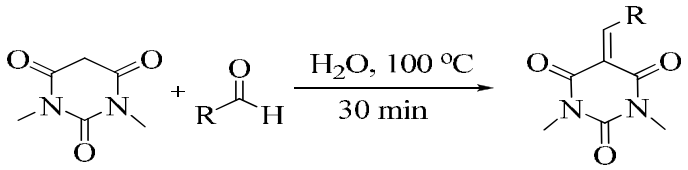
Results and Discussion

Chemistry

5-Arylidene-*N,N*-dimethylbarbiturates **1-22** were re-synthesized using same procedure as described previously and physical as well as spectroscopic data of all compounds was completely in agreement with the reported data (Scheme-1) [17].

Antibacterial Studies

All the synthetic arylidene *N,N*-diethylthiobarbiturate **1-22** were screened against twelve Gram-positive *i.e. Bacillus cereus*, *Bacillus subtilis, Bacillus thuringiensis*, *Cornybacterium diptheriae, Corynebacterium hoffmanii*, *Corynebacterium xerosis*, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus*, *Staphylococcus aureus* AB 188, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Streptococcus faecalis*, and twelve Gram-negative bacterial strains *i.e.*, *Enterobacter*, *Escherichia coli* ATCC 8739, *Escherichia coli*, *Escherichia coli* (MDR), *Klebsiella pneumonia, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* ATCC 9027, *Shigella dysenteriae*, *Salmonella typhi, Salmonella paratyphi* A, and *Salmonella paratyphi* B. Results of antibacterial studies are shown in Table-1 and 2.



1: R = 4-$(CH_3)_2N$-C_6H_5-
2: R = 4-OH-C_6H_4-
3: R = 3, 4-*di*-Cl-C_6H_3-
4: R = 4-CH_3S-C_6H_4-
5: R = 4-NO_2-C_6H_4-
6: R = 2,3,4-*tri*-OH-C_6H_2-
7: R = 2, 4-*di*-Cl-C_6H_3-
8: R = 2-OH, 3-C_2H_5O-C_6H_3-
9: R = 2-OH, 3-CH_3O-C_6H_3-
10: R = 3, 4-*di*-OH-C_6H_3-
11: R = 2-NO_2-C_6H_4-
12: R = 2, 4-*di*-OH-C_6H_3-
13: R = 2-OH, 5-CH_3-C_6H_3-
14: R = 3-NO_2-C_6H_4-
15: R = 3-C_2H_5O, 4-OH-C_6H_3-
16: R = 3,4-*di*-CH_3O-C_6H_3-
17: R = 4-Br-C_6H_5-
18: R = 3-Cl-C_6H_4-
19: R = 3,4,5-*tri*-CH_3O-C_6H_2-
20: R = 2-C_2H_5O-C_6H_5-
21: R = 4-C_2H_5O-C_6H_5-
22: R = C_6H_5-

Scheme-1: Synthetic route for 5-Arylidene-*N,N*-Dimethylbarbiturates Derivatives.

Table-1: *In vitro* Antibacterial activity (Gram-positive organisms) (Zone of inhibition in mm).

Compound No.	*Bacillus cereus*	*Bacillus subtilis*	*Bacillus thuringiencis*	*Corynebacterium diptheriae*	*Corynebacterium hofmani*	*Corynebacterium xerosis*	*Micrococcus luteus* ATCC 9341	*Staphylococcus aureus*	*Staphylococcus aureus* AB 188	*Staphylococcus epidermidis*	*Staphylococcus Saprophyticus*	*Streptococcus faecalis*
1	-	-	**8**	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	**8**	**13**	-	**8**	-	**10**	**9**	-	**10**	**15**	**15**	**13**
6	**8**	-	**10**	-	-	-	**8**	-	**10**	**15**	-	**7**
7	-	-	-	**8**	**8**	-	**7**	-	**8**	**8**	-	**7**
8	**8**	-	**8**	**9**	**8**	**8**	**7**	-	-	-	-	-
9	**12**	-	-	**8**	**7**	-	**7**	-	**8**	**9**	-	-
10	**7**	-	-	-	-	**7**	**12**	-	-	**12**	-	-
11	**15**	**15**	-	**12**	**15**	**12**	**18**	-	**15**	**20**	**15**	**9**
12	-	-	-	**8**	-	-	-	-	-	-	-	**22**
13	-	-	-	**7**	-	-	-	-	-	-	-	**18**
14	**7**	-	**10**	**8**	-	-	**11**	-	**9**	-	**11**	**10**
15	-	-	-	-	-	-	**7**	-	-	-	**10**	**16**
16	-	-	-	-	**7**	**7**	-	-	-	-	**8**	**14**
17	-	-	-	-	**7**	**7**	-	-	-	-	**11**	**15**
18	-	-	-	**7**	-	-	-	-	-	-	**9**	**19**
19	-	-	-	**8**	-	-	-	-	-	-	**9**	**14**
20	**9**	-	-	**7**	-	**10**	-	-	-	-	**8**	**17**
21	-	-	-	**7**	-	-	-	-	-	-	**8**	**14**
22	-	-	-	**9**	-	**7**	-	-	-	-	**11**	**24**

Table-2: *In vitro* Antibacterial activity (Gram-negative organisms) (Zone of inhibition in mm).

Compound No.	*Enterobacter*	*Escherichia coli* ATCC 8739	*Escherichia coli*	*Escherichia coli* multi drug resistant	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeroginosa*	*Pseudomonas aeroginosa* ATCC9027	*Shigella dysenteriae*	*Salmonella typhi*	*Salmonella paratyphi* A	*Salmonella paratyphi* B
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	**10**	-	-	**11**	-	-	-	-	-	**13**	**7**
6	**9**	**12**	-	**9**	**9**	**10**	-	**7**	-	-	-	**7**
7	-	-	-	**7**	-	-	-	-	-	-	-	**7**
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	**7**	-	-	-	-	**9**	-	-
10	-	-	-	-	-	-	-	-	-	-	-	**8**
11	-	-	-	-	-	-	-	-	-	-	**11**	-
12	-	-	**13**	-	-	-	-	-	-	**8**	-	**7**
13	-	-	-	-	**7**	-	-	**7**	-	-	-	**7**
14	-	-	-	-	**7**	-	**7**	**11**	**9**	-	**10**	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	**7**	-	-	-	-
17	-	-	-	-	**8**	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	**7**	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-

Compound **1** was found to be inactive against all Gram-positive and Gram-negative bacterial strains except weakly active against *Bacillus thuringiensis.* Compound **2-4** were found to be inactive against all tested bacterial strains. Compound **5** found to be moderately active against all Gram-positive bacterial strains except *Bacillus thuringiensis, Corynebacterium hoffmanii* and *Staphylococcus aureus,* however, against Gram-negative bacterial strains, compound **5** showed weak activity *i.e. Escherichia coli* ATCC 8739, *Klebsiella pneumonia, Salmonella paratyphi* A, and *Salmonella paratyphi* B. Compound **6** was found to be moderate active against six Gram-positive and seven Gram-negative bacterial strains. Compounds **7-10** showed good activity against most of Gram-positive strain but inactive against most of Gram-negative bacterial strains. Compound **11** showed excellent activity against Gram-positive bacterial strains, nevertheless, inactive against Gram-negative bacterial strains, compound **11** showed activities only against *Salmonella paratyphi* A. Rest of the compounds showed weak activity against some of Gram-positive and Gram-negative tested bacterial strains.

Anti-Fungal Activity

Compounds **1-22** were tested against fifteen fungal strains *i.e.*, *Aspergillus flavus, Aspergillus niger, Fusarium specie, Helminthospourm, Penicillium specie, Rhizopus, Candida albican, Candida albican* ATCC 0383, *Saccharomyces cereviseae, Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes,* and *Trichophyton tonsurans.* Results of antifungal studies are shown in Table-3.

Table-3: *In vitro* Anti-Fungal activity (Zone of inhibition in mm).

Compound No.	*Asppergillus flavus*	*Asppergillus niger*	*Fusarium* specie	*Helminthospourm*	*Penicillium* specie	*Rhizopus*	*Candida albican*	*Candida albican ATCC 0383*	*Saccharomyces cereviseae*	*Microsporum canis*	*Microsporum gypseum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Trichophyton tonsurans*
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	**10**	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	**7**	-	-	-	**7**	-	-	**11**	**14**	-	-	-	-	-
6	-	-	-	-	-	-	-	-	**8**	-	-	-	-	-
7	-	-	-	-	-	-	-	-	**9**	-	-	-	-	-

Table-3: continue…

8	**7**	-	-	-	**8**	-	-	**14**	**11**	**8**	-	-	-	-
9	-	-	-	-	-	-	**11**	**15**	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	**8**	-	-	-	**7**	-	-	**8**	**11**	-	-	-	-	**8**
12	-	-	-	-	-	-	-	-	-	-	**7**	-	-	-
13	-	-	**8**	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	**12**	-	-	**8**	-	-	-
15	-	-	-	-	-	-	-	-	**7**	-	-	-	-	-
16	-	-	-	-	-	-	-	-	**9**	-	-	-	-	-
17	-	-	-	-	-	-	-	-	**9**	-	**8**	-	-	-
18	-	-	-	-	-	-	-	-	**8**	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	**8**	-	-	-	-	-
22	-	-	-	-	-	-	-	-	**9**	-	-	-	-	-

Compounds **1, 3, 4, 10, 19** and **20** were found to be inactive against all antifungal strains. Compound **5** was active against *Aspergillus flavus, Penicillium specie, Candida albican* ATCC 0383*,* and *Saccharomyces cereviseae.* Compound **8** was found to be active against *Aspergillus flavus, Penicillium specie, Candida albican* ATCC 0383, *Saccharomyces cereviseae* and *Microsporum canis.* Compound **9** showed activity against *Candida albican* and *Candida albican* ATCC 0383, while compound **11** was found to be moderately active against *Aspergillus flavus, Penicillium specie, Candida albican* ATCC 0383, *Saccharomyces cereviseae,* and *Trichophyton tonsurans.* Compounds **12** and **13** were feebly active against *Microsporum gypseum* and *Fusarium specie,* respectively. Compound **14** showed activity against *Candida albican* ATCC 0383 and *Microsporum gypseum* while compound **17** showed activity against *Saccharomyces cereviseae* and *Microsporum gypseum.* Compounds **2**, **6**, **7**, **15**, **16**, **18**, **21** and **22** were active only against *Saccharomyces cereviseae.*

Preparation of Sterile Discs

Filter paper discs of 6 mm were prepared and then autoclaved at 121°C for 15 minutes. 10 μL of test compound containing 100 μg was applied onto the sterile discs and applied onto the surface of medium seeded with microorganisms.

Preparation of Bacterial Inoculum

Mueller Hinton Broth (Oxoid, Hampshire, England)) tubes were inoculated with different bacterial cultures. These inoculated tubes were kept in shaking incubator for 2 h at 37 °C to get bacterial log phase cultures. After 2 h, these tubes were vortex to get homogenize bacterial suspension and matched with 0.5 McFarland's standard (Mc Farland & Jama, 1907) to get approximately 1-2 x 10^8 CFU/ml.

Preparation of Fungal Inoculum

Large amount of fungal cultures were scrapped from stock slants and transferred in screw capped tubes containing sterile saline/distilled water and glass beads and then these tubes were vortex enough for homogenization, especially for dermatophytes.

Anti-Bacterial Bioassay (In vitro)

Disc Diffusion Method (Bauer *et al.,* 1966) [19] was followed to determine the antibacterial activity. Briefly, sterile cotton swab was dipped in standardized bacterial suspension then cotton swab was rotated firmly against the upper inside well of the tube to remove excess fluid. Entire agar surface of the plate was streaked with the swab three times turning the plate 60° angle between each streaking. The plates were allowed to dry and then discs impregnated with stock solutions of different compounds were placed on to the agar surface. A disc loaded with 10 μL of DMSO was placed on plates as a control. The plates were incubated at 37 °C in incubator for 24 h in inverted position. The area around disc free from bacterial growth was measured in mm as zone of inhibition and recorded.

Anti-Fungal Bioassay (In vitro)

Antifungal activity was also determined by Disc diffusion method. Sabouraud's Dextrose agar (Oxoid, Hampshire, England) was used as a growth medium. Fungal lawn was made on media plates. The plates were allowed to dry and then discs impregnated with compounds were placed onto the agar surface. A disc loaded with 10 μL of DMSO was placed on plates as a control. The plates were incubated at room temperature and examined for their antifungal activity after 3-7 days. The area around disc free from fungal growth was measured in mm as zone of inhibition and recorded.

Experimental

EIMS were recorded with MAT 711 (70 eV) spectrometer. ^{1}H-NMR spectra were recorded with Bruker AC400 (400 MHz) spectrophotometer. Reactions progress was monitored by TLC (Merck). The chromatograms were visualized under ultraviolet light.

General Procedure for the Synthesis of 5-arylidene Barbiturates **1-22**

N,N-Dimethylbarbituric acid (1.0 mmol) and corresponding aldehyde (1.00 mmol, 1 eq.) were refluxed in distilled water for 30 minutes. In all cases, solid products were formed which were filtered and washed with cold water and ether followed by drying under vacuum [17].

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