Xanthine Oxidase Inhibition by 5-aryledene *N,N'*-dimethylbarbituric Acid Derivatives

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Summary: *N,N'*-dimethylbarbituric acid derivatives **1-24** were evaluated for their xanthine oxidase (XO) inhibitory activity. Majority of these compounds showed a good to moderate *in vitro* xanthine oxidase inhibitory activity (IC_{50} = 20.97 ± 0.29 - 327.0 ± 3.50 μM), while eight compounds were found to be completely inactive. A structure-activity relationship has been discussed, identifying structural features, responsible for varying degree of activity.

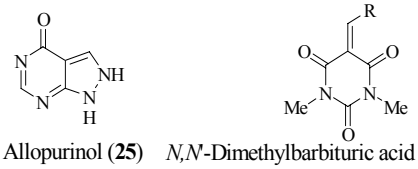
Keywords: Xanthine oxidase inhibition, *N,N*'-dimethylbarbituric acid derivatives, gout, antioxidants.

Introduction

Xanthine oxidase (XO) catalyzes the hydroxylation of hypoxanthine and xanthine, last two steps in the formation of urate. During the past decade, numerous studies have suggested that XO plays an important role in ischemic and other types of tissue and vascular injuries, chronic heart failure, hyperuricemia and inflammatory diseases [1-4]. The hyperuricemia can lead to hypertension, hyperlipidaemia, cancer, diabetes, and obesity [5]. The most effective treatment of gout is reduction in the uric acid production by inhibition of XO or increasing the excretion of uric acid. The xanthine oxidase (XO) inhibitors are not only useful, but they also possess lesser side effects as compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically used XOI since last three decades, which unfortunately suffers from some adverse effects, such as renal toxicity, hypersensitivity syndrome and Stevens-Johnson syndrome [6]. Therefore the search for new and safe XOI for the treatment of gout and various other diseases is urgently required.

The first barbituric acid was synthesized in 1864 by Adolph von Baeyer [7]. Word "barbiturate" is based on the combination of the words Barbara and urea [7]. Barbituric acid itself is hypnotically inactive, but substitution at C-5 of the barbiturate ring makes these analogs active as central nervous system depressants. Diethylbarbituric acid, the first hypnotically active barbiturate, was synthesized in 1903 [8]. Since then over 2,500 barbiturates have been synthesized [9, 10]. Pentobarbtione and thiopentone were synthesized in 1930 and 1932, respectively [11]. Seventy years later, they remain the two most commonly used barbiturates for the management of acute neurological and neurosurgical emergencies. Basically, barbiturates exhibit their anesthetic as well as sedative properties by enhancing the action of c-aminobutyric acid (GABA) at the GABAA receptor [12-18]. *In vitro* neuroprotective effects including inhibition of presynaptic glutamate release [19, 20], attenuation of post-synaptic glutamate activity at NMDA and AMPA-receptors [19, 21], calcium accumulation inhibition in synaptosomes [22], and nitric-oxide induced cytotoxicity inhibition [23].

In the present study, we synthesized a series of *N,N'*-dimethylbarbituric acid derivatives **1-24**, structurally close to allopurinol (**25**), with one of its rings resembled to barbituric acid (Fig. 1). These derivatives were then screened for their *in vitro* xanthine oxidase (XO) inhibitory activity, and some encouraging results are obtained



Allopurinol (**25**) *N,N'*-Dimethylbarbituric acid derivatives **1-24**

Fig. 1: Resemblance of barbituric acid derivatives (1-24) with allopurinol (25)

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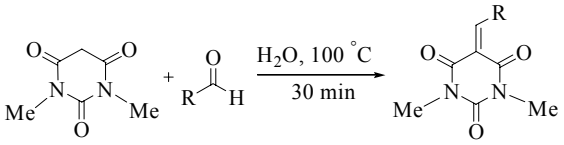
Results and Discussion

Chemistry

N,N'-Dimethylbarbituric acid derivatives **1-24** were prepared from *N,N'*-dimethylbarbituric acid by condensing with appropriate aromatic aldehydes in water under reflux conditions in high yields (**Scheme-1**). In a typical reaction, Appropriated aldehydes (1.56 mmol, 1 eq.) and *N,N'*-dimethylbarbituric acid (0.2 g, 1.56 mmol) were suspended in distilled water (10 mL) at room temperature. This mixture was heated up to reflux for 30 minutes with continuous monitoring through TLC. When reaction was completed (TLC analysis), solid products were filtered. The crude products were washed with cold water and then ether. After washing, the solid products were dried in a desiccator under vacuum and collected as fluffy solids. The structures of **1-24** were elucidated by using different spectroscopic techniques *i.e.* ^{1}H-NMR and EI-MS [24].



Scheme-1: Synthetic route for barbituric acid derivatives **1-24**.

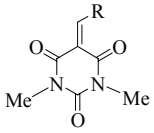
Bioactivity

Xanthine oxidase (XO) is involved in metabolic pathway towards uric acid formation. XO can act on certain purines, pterins, and aldehydes [25]. It can efficiently catalyzes the conversion of 1-methylxanthine (a metabolite of caffeine) to 1-methyluric acid, but has low activity on 3-methylxanthine. The xanthine oxidase (XO) inhibitor, allupurinol, is used in the treatment of gout [26].

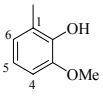
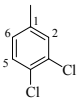
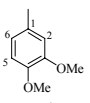
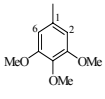
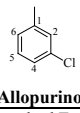
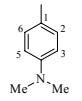
Based on the structure of allopurinol, *N,N'*-dimethylbarbituric acid derivatives **1-24** were synthesized and screened for their xanthine oxidase inhibitory potential. The basic hypothesis was that an amide bond and a six member aromatic ring in the allupurinol and barbituric acid, respectively, may exhibit inhibitory potential against the xanthine oxidase. Amongst the *N,N'*-dimethylbarbituric acid derivatives **1-24**, sixteen showed a good to moderate xanthine oxidase inhibiting potential with IC_{50} values in the range of 20.97 ± 0.29 - 327.0 ± 3.50 μM, while eight were found to be completely inactive.

Limited SAR suggests that the XO inhibitory activity of **1-24** largely depends on the substitution on phenyl ring and other structural features. Compound **16** without any substitution on phenyl residue showed an IC_{50} value 327.0 ± 3.5 μM, and found to be least active among the sixteen active derivatives.

Table-1: Synthetic derivatives 5-aryledene *N,N'*-dimethyl barbituric acid **(1-24).**



1-24

Comp. No.	R	IC_{50} ± SEM[a] μM	Comp. No.	R	IC_{50} ± SEM[a] μM
1		**20.97 ± 0.29**	**13**		**196.34 ± 1.01**
2		**24.25 ± 0.50**	**14**		**233.8 ± 5.50**
3		**26.94 ± 1.01**	**15**		**278.8 ± 1.41**
4		**41.00 ± 1.50**	**16**		**327.0 ± 3.50**
5		**70.19 ± 1.8**	**17**		**NA**
6		**71.49 ± 0.53**	**18**		**NA**
7		**75.03 ± 2.00**	**19**		**NA**
8		**93.70 ± 2.30**	**20**		**NA**
9		**98.90 ± 1.01**	**21**		**NA**
10		**118.22 ± 0.84**	**22**		**NA**
11		**120.5 ± 0.19**	**23**		**NA**
12		**125.5 ± 2.05**	**24**		**NA**
25	**Allopurinol**	**13.70 ± 0.15**			

[a]SEM = Standard Error of Mean

The most active compound **1** with an IC_{50} value 20.97 ± 0.29 μM possess two chloro residues at *ortho* and *para* positions of the phenyl ring of the barbituric acid. Comparing the activity of compound **1** with the other dichloro substituted (*meta* and *para*) compound **9** (IC_{50} = 98.90 ± 1.01 μM) indicated that the change in the position of one of the chloro groups (*ortho* to *meta*) sharply decrease the XO inhibitory activity. On the other hand, elimination of one of the chloro groups from *ortho* position as in compounds **12** (IC_{50} = 125.5 ± 2.05 μM) and **13** (IC_{50} = 196.34 ± 1.01 μM) also decrease the XO inhibitory activity. This difference in activity of compounds **1**, **9**, **12**, and **13** suggested that the position and nature of substituent at phenyl ring are responsible for varying activity. By changing the *para*-chloro of compound **13** with *para*-bromo group, as in compound **7** (IC_{50} = 75.03 ± 2.00 μM), increases the inhibitory potential. Lack of access to suitable precursors to synthesize other bromine substituted compounds did not allow us to study SAR of bromo derivatives.

Compound **2** (IC_{50} = 24.25 ± 0.50 μM) found to be second most active compound of the series with *ortho*, *meta*, and *para* tri-hydroxyl phenyl residues, suggesting that three hydroxyl groups may be responsible for xanthine oxidase inhibitory activity. When one of the hydroxyl groups was eliminated, as in compounds **17** and **18**, both of them lost their inhibitory potential, indicating that -OH at *ortho*, *meta* and *para* positions are essential for the activity of the molecules. When *ortho* and *meta* positions of phenyl ring were substituted with hydroxyl and ethoxy groups, respectively, as in compound **4**, it showed a good inhibitory potential (IC_{50} = 41.00 ± 1.50 μM). However, when in compound **19**, OH group moved from *ortho* position, to *para* position, maintaining the ethoxy group at *meta* position, a total loss of activity was observed. In compound **8**, when ethoxy was substituted with a OCH_3 residue, a reduction in activity was observed (IC_{50} value 93.70 ± 2.30 μM), as compared to its parent compound **4**, hence indicating that replacement of a OH along with a OCH_3 group at a suitable position play a role in the activity. When we compared the activity of compound **14** with *ortho* hydroxyl and *meta* methyl, a very weak inhibitory activity was observed (IC_{50} = 233.8 ± 5.50 μM) further indicating the importance of the hydroxyl at a suitable position. This initial inference was also supported by compound **15** with only ethoxy substitution at *ortho* position (IC_{50} = 278.8 ± 1.41 μM) displaying a very weak activity, as compared to compound **4**. The activity of compound **15** also indicates that the position of ethoxy is also an important contributor to activity. When ethoxy group is shifted to *para* position, as in compound **20**, activity is totally lost. When phenyl was replaced with the bicyclic naphthalene, a good activity (IC_{50} = 120.5 ± 0.19 μM) was observed. Compounds **21** and **22**, with two and three methoxy substituents, demonstrated no activity against the enzyme. *Para* sulfide containing compound **23** and *para N,N'*-dimethyl substituted phenyl compound **24** were also found to be inactive.

Compound **3** with an *ortho*-nitro on phenyl ring showed a lower IC_{50} = 26.94 ± 1.01 μM, as compared to its *para* nitro analog (compound **5**) (IC_{50} = 70.19 ± 1.8 μM), while the nitro group at *meta* position (compound **6**) leads to a slight decrease in activity (IC_{50} = 71.49 ± 0.53 μM), suggesting that -NO_2 at suitable disposition contributes in activity.

This limited structure-activity relationship (SAR) showed that the substituents on phenyl ring (nitro, chloro, bromo, hydroxyl and ethoxy) play important role in the xanthine oxidase enzyme inhibition potential of *N,N'*-dimethylbarbituric acid derivatives.

Experimental

Xanthine Oxidase Inhibition Assay In Vitro

The XO inhibitory activity of test compounds was determined by measuring the rate of hydroxylation of the substrate (xanthine) into uric acid, which is a colorless end product of the reaction and shows absorption at 295 nm [25]. Briefly, the reaction mixture containing 10 μL of 1 mmol/L pure sample was dissolved in DMSO, 150 μL of phosphate buffer (0.05 mol/L, pH 7.4), 0.003 units of xanthine oxidase dissolved in buffer (20 μL), and 20 μL of 0.1 mmol/L xanthine as substrate for enzyme. After addition of xanthine oxidase, the mixture was incubated for 10 min at room temperature and pre-read in the UV region (λ max 295 nm). The substrate was added to the reaction mixture, and final readings were carried out for 15 min at an interval of 1 min (Spectra MAX-340). The percentage inhibitory activity by the samples were determined against a DMSO blank, and calculated by using the following formula.

Inhibition (%) = 100 - [(OD test compound /OD control) × 100]

The IC_{50} of the compounds was calculated by using EZ-Fit windows-based software (Perrella Scientific Inc. Amherst, USA). To compare the inhibitory activities of the compounds, allopurinol (Sigma/Aldrich Catalogue # A8003) was used as

standard and each compound was assayed in triplicate.

General Procedure for the Synthesis of 5-arylidene Barbiturates **1-24**

N,N'-Dimethyl barbituric acid (1.56 mmol) and corresponding aldehyde (1.56 mmol, 1 eq.) were dissolved in 10 mL of distilled water and the mixture was refluxed for 30 minutes. In all cases, solid product were formed which were filtered, washed with cold water and ether and dried under vacuum. The pure compounds **1-24** were obtained as fluffy solids having satisfactory physical and spectroscopic data [24].

Conclusion

Using allopurinol as the minimum pharmacophore, a library of twenty-four derivatives was evaluated for their *in vitro* xanthine oxidase inhibitory activity. Out of them, sixteen *N,N'*-dimethylbarbituric acid derivatives showed a good to moderate *in vitro* xanthine oxidase inhibitory activity, while eight compounds were found to be completely inactive. Conclusively, current study suggests that *N,N'*-dimethylbarbituric acid derivatives may have potential to inhibit xanthine oxidase enzyme, however, an extensive work in connection of refining the structures of these molecules is required.

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References

1. C. E. Berry and J. M. Hare, *Journal of Physiology* (*London*), **555**, 589 (2004).
2. R. Harrison, *Free Radical Biology and Medicine*, **33**, 774 (2002).
3. K. M. Khan, N. Ambreen, U. R. Mughal, S. Jalil, S. Perveen, M. I. Choudhary, *European Journal of Medicinal Chemistry* **45**, 4058 (2010).
4. R. Harrison, *Drug Metabolism Reviews*, **36**, 363 (2004).
5. K. C. Lin, H.Y. Linm and P. Chou, *Journal of Rheumatology*, **27**, 1501 (2000).
6. A. Burke, E. Smyth, G. A. FitzGerald, Analgesic–antipyretic agents; pharmacotherapy of gout. In: L. L. Brunton, J. S. Lazo, K. L. Parker, (Eds.), The pharmacological basis of therapeutics, 11th ed. McGraw. Hill Medical Publishing Division, New York, 2006, pp. 706-710.
7. J. W. Dundee and P. D. A. McIlroy, *Anaesthesia*, **37**, 726 (1982).
8. S. C. Harvey, Hypnotics and sedatives. The barbiturates. In: L. S. Goodman, A. Gilman (eds) The pharmacological basis of therapeutics. 5th ed. Macmillan, New York (1975) p.102.
9. a) K. M. Khan, M. Ali, T. A. Farooqui, M. Khan, M. Taha and S. Perveen, *Journal of Chemical Society of Pakistan*, **31**, 823 (2009). b) S. Perveen, S. Mustafa and K. M. Khan, *Journal of Chemical Society of Pakistan*, **32**, 229 (2010).
10. M. Willow and G. A. R. Johnston, *International Review of Neurobiology*, **24**, 15 (1983).
11. J. W. Dundee, *Br. Journal of Anaesthesia*, **56**, 211 (1984).
12. N. Akaike, K. Hattori, N. Inomata and Y. Oomura, *Journal of Physiology*, **360**, 367 (1985).
13. N. Akaike, T. Maruyama and N. Tokutomi, *Journal of Physiology*, **394**, 85 (1987).
14. J. M. H. French-Mullen, J. L. Barker and M. A. Rogawski, *Journal of Neurosciences*, **13**, 3211 (1993).
15. L. Y. M. Huang and J. L. Barker, *Science*, **207**, 195 (1980).
16. F. Leeb-Lundberg and R. W. Olsen, *Molecular Pharmacology*, **21**, 320 (1982).
17. J. A. Peters, E. F. Kirkness, H. Callachan, J. Lambert and A. J. Turner, *Br. Journal of Pharmacology*, **94**, 1257 (1988).
18. S. A. Thompson, P. J. Whiting and K. A. Wafford, *British Journal of Pharmacology*, **117**, 521 (1996).
19. R. L. Macdonald and M. J. McLean, *Epilepsia*, **23**, 7 (1982).
20. M. B. Waller and J. A. Richter, *Biochemical Pharmacology*, **29**, 2189 (1980).
21. H. Zhu, J. E. Cottrell and I. S. Kass, *Anesthesiology*, **87**, 944 (1997).
22. M. P. Blaustein, A. C. Ector, *Molecular Pharmacology*, **11**, 369 (1975).
23. S. Shibuta, J. Kosaka, T. Mashimo, Y. Fukuda and I. Yoshiya, *British Journal of Pharmacology*, **124**, 804 (1998).
24. K. M. Khan, M. Khan, M. Ali, M. Taha, A. Hameed, S. Ali, S. Perveen and M. I. Choudhary, *Medicinal. Chemistry*, **7**, 231 (2011).
25. M. A. Whidden, J. M. McClung, D. J. Falk, M. B. Hudson, A. J. Smuder, W. B. Nelson and S. K. Powers, *Journal of Applied Physiology*, **106**, 385 (2009).
26. L.D. Kong, Y. Cai, W. W. Huang, C. H. Cheng and R. X. Tan, *Journal of Ethnopharmacology*, **73**, 199 (2000).
27. S. K. Lee, Z. H. Mbwambo, H. Chung, L. Luyengi, E. J. Gamez and R. G. Mehata, *Combinatorial Chemistry and High Throughput Screening*, **1**, 35 (1998).