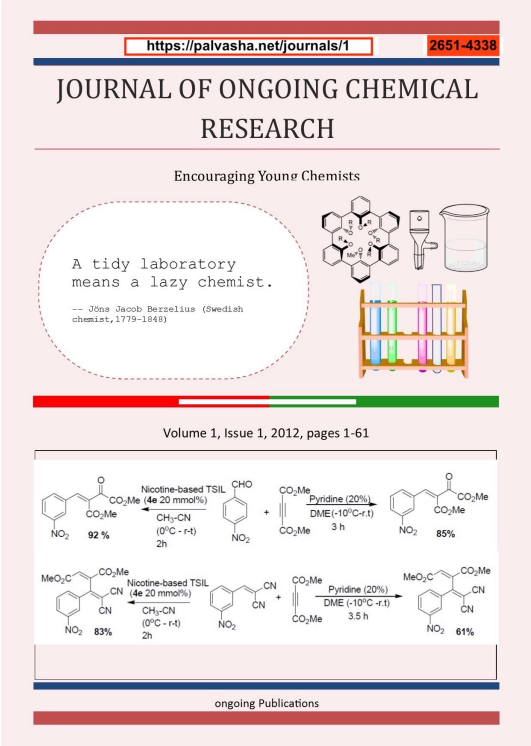
SYNTHESIS AND MOLECULAR DOCKING STUDY OF *BIS*-THIOBARBITURATE DERIVATIVES AS EFFECTIVE INHIBITORS OF BETA-GLUCURONIDASE



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Research Article

Synthesis and Molecular Docking Study of *bis*-thiobarbiturate Derivatives as Effective Inhibitors of Beta-glucuronidase

Hayat Ullah, Fahad Khan, Fazal Rahim, Zia Ur Rehman, Muhammad Taha, Abdul Wadood, Fawad Ahmad, Zainul Wahab, Imad Uddin*, Khalid Zaman
For affiliations and correspondence, see the last page.

Abstract

Bis-thiobarbiturate derivatives **1-15** have been synthesized and evaluated for their *in vitro* β-glucuronidase inhibitory potential. The structures of all compounds were confirmed through spectroscopic techniques such as EI-MS and ^{1}HNMR. Compounds **13** (IC_{50} = 29.42 ± 0.61μM) showed potent β-glucuronidase inhibitory potential better than the standard inhibitor (D-saccharic acid 1, 4 lactone, IC_{50} = 48.4 ± 1.25 μM). The compounds **2** (IC_{50} = 48.45 ± 0.39 μM), **3** (IC_{50} = 53.12 ± 0.22 μM), **4** (IC_{50} = 55.12 ± 1.13μM) and **9** (IC_{50} = 49.84 ±1.46μM) showed *b*-glucuronidase inhibitory potential comparable to the standard inhibitor. Compounds **1** (IC_{50} = 68.45 ± 0.33 μM), **5** (IC_{50} = 61.18 ± 1.42μM), **6** (IC_{50} = 92.57 ±2.80 μM), **7** (IC_{50} = 61.27 ± 0.45 μM), **8** (IC_{50} = 62.85 ± 0.79μM), and **12** (IC_{50} = 77.56 ± 1.32μM) also showed good inhibitory potential. The remaining compounds are found to be completely inactive. The structure-activity relationship was established for these compounds. This study identified a novel class of β-glucuronidase inhibitors.

Keywords: Organic Synthesis, Molecular Docking Studies, Thiobarbituric Acid, β-glucuronidase Inhibition, Thiobarbiturate

INTRODUCTION

β-Glucuronidase (EC 3.2.1.31) is an inducible enzyme found in anaerobic *Escherichia, Bacteroides*, *Clostridia* and *Peptostreptococcus* genera that catalyzes the cleavage of β-glucuronosyl-O-bonds [Sperker et al, 1997]. The enzyme exists in many human body fluids and organs such as bile, kidney, serum, spleen and urine. β-Glucuronidase has enhanced activity in a variety of pathological conditions including epilepsy [Plum, 1967], renal diseases [Gonick et al, 1973], urinary tract infection [Ronald et al, 1971], transplantation rejection [Schapiro et al, 1968] and neoplasm of bladder [Hradec et al, 1965], breast, larynx and testes [Boyland et al, 1957]. Moreover, β-glucuronidase has been reported to be released in the synovial fluid in the inflammatory joint diseases, for instance, rheumatoid arthritis [Weissmann et al, 1971]. The over-expression of the enzyme is also reported in some hepatic diseases and AIDS. The involvement of β-glucuronidase in the colon cancer and higher intestinal levels of the enzyme is correlated to the higher incidence of colon carcinoma [Goldin et al, 1976]. This observation is supported from the fact that the administration of a bacterial β-glucuronidase inhibitor lead to a decrease in carcinogen induced colonic tumors. These reports clearly suggest that the development of specific inhibitors of β-glucuronidase has great pharmacological importance.

Thiobarbituric acid is different from barbituric acid due to presence of a sulfur atom instead of an oxygen atom. Two active methylene hydrogen atoms at carbon-5 flanked between the two carbonyl carbons due to which their acidity further increased and their derivatives show significance biological activities [Zuccarello et al, 2003]. Thiobarbituric acid analogs have been also reported as Antifungal [Kidwai et al, 2005], antidepressant [Singh et al, 1992], antimicrobial [Essa et al, 2012], anti-tubercular [Ralhan et al, 1960], herbicides [Alimmari et al, 2010], anti-convulsing [Srivastava et al, 2004], anti-sclerosis's [Khan et al, 2009] and matrix metalloproteinase. Thiobarbituric acid analogs also showed anti-cancer and anti-viral activities.

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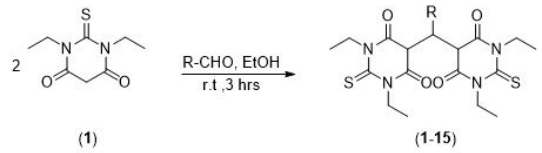

Figure 1. Synthesis of thiobarbituric acid analogs (**1-15**

In continuation of our ongoing research on the chemistry and bioactivity of new heterocyclic compounds [Rahim et al, 2015], we carried out the synthesis of *bis*-thiobarbiturate derivatives, which is reported herein.

RESULTS AND DISCUSSION

Chemistry

The *bis*-thiobarbiturate derivatives **1-15** have been synthesized by the reaction of 1,3-diethyl-2-thioxodihydropyrimidine-4,6-(1*H*, 5*H*)-dione (*N,N*-diethylthiobarbituric acid, 2 mmol) with different aromatic aldehydes (1 mmol) in the presence of 5-10 mL of EtOH. Reaction mixture was stirrer in ethanol about 3 hrs. The reaction completion was monitored by periodic TLC. After completion of reaction the mixture was poured into crushed ice followed by acidification with dil. HCl. Solid precipitates was obtained, filter, dried and recrystallized from ethanol give pure product in excellent yield [Rahim et al, 2016]. The structures of all synthetic compounds **1-15** (Figure 2) were confirmed through EI-MS and ^{1}HNMR. Figure 1 shows the synthetic scheme.

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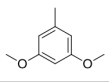
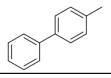
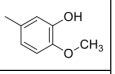
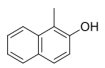
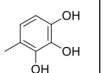
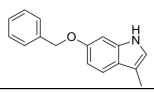
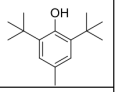
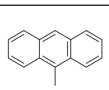
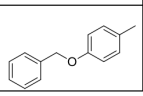
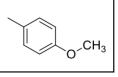
1		6		11	
2		7		12	
3		8		13	
4		9		14	
5		10		15	

Figure 2. Various substituents of thiobarbituric acid derivatives (**1-15**)

In vitro β-Glucuronidase Inhibitory Potential

Bis-thiobarbiturate derivatives **1-15** have been synthesized and evaluated for their *in vitro* β-glucuronidase inhibitory potential. Compounds **13** (IC_{50} = 29.42 ± 0.61μM) showed potent β-glucuronidase inhibitory potential better than the standard inhibitor (D-saccharic acid 1, 4 lactone, IC_{50} = 48.4 ± 1.25 μM). The compounds **2** (IC_{50} = 48.45 ± 0.39 μM), **3** (IC_{50} = 53.12 ± 0.22 μM), **4** (IC_{50} = 55.12 ± 1.13μM) and **9** (IC_{50} = 49.84 ±1.46μM) showed *b*-glucuronidase inhibitory potential comparable to the standard inhibitor. Compounds **1** (IC_{50} = 68.45 ± 0.33 μM), **5** (IC_{50} = 61.18 ± 1.42μM), **6** (IC_{50} = 92.57 ±2.80 μM), **7** (IC_{50} = 61.27 ± 0.45 μM), **8** (IC_{50} = 62.85 ± 0.79μM), and **12** (IC_{50} = 77.56 ± 1.32μM) also showed good inhibitory potential. The remaining compounds are found to be completely inactive (Table 1).

Table 1. Result of β-glucuronidase activity of *Bis*-thiobarbiturate derivatives **1-15**

S. No.	IC_{50} ± SEM[a] (μM)	S. No.	IC_{50} ± SEM[a] (μM)
1	68.45 ± 0.33	**9**	49.84 ±1.46
2	48.45 ± 0.39	**10**	NA[b]
3	53.12 ± 0.22	**11**	NA[b]
4	55.12 ± 1.13	**12**	77.56 ± 1.32
5	61.18 ± 1.42	**13**	29.42 ± 0.61
6	92.57 ±2.80	**14**	NA[b]
7	61.27 ± 0.45	**15**	NA[b]
8	62.85 ± 0.79	D-saccharic acid 1,4-lactone,	48.4 ± 1.25

The structure activity relationship has been carried out for all compounds. The SAR is mainly based on change in the substitution pattern of aromatic aldehyde. The *N,N*-dimethyl amino substituted analog **13** showed outstanding inhibitory activities among the series with IC_{50} values of 29.42 ± 0.61μM. The quinoline substituted analog **2** having IC_{50} value (48.45 ± 0.39 μM) got second position among the series, and indolyl analog **3** having IC_{50} value (53.12 ± 0.22 μM). Interestingly all the three compounds **13, 2** and **3** have extra nitrogen with in the basic skeleton, and the activity might be due to this extra nitrogen atom with in the scaffold. The slight activity difference might be due to steric hindrance. The *m*-hydroxy analog **9,** 3,4-dihydroxy analog **4,** 2-naphthol substituted analog **7,**

p-hydroxy analog **8** and 2,3,4-trihydroxy analog **12** having IC_{50} values 49.84 ±1.46, 55.12 ± 1.13, 61.27 ± 0.45, 62.85 ± 0.79 and 77.56 ± 1.32 μM respectively. Among the hydroxyl analogs the *m*-hydroxy analog is found to be most active. In this study it was observed that among hydroxyl analogs the number as well as position of hydroxyl group on the phenyl is might be responsible for this inhibition. As the number of hydroxyl groups are increase on phenyl ring, the activity become decreases. The reason for this might be steric hindrance. The anthracenyl analog **5,** 3,5-dimethoxy analog **1** and biphenyl analog **6** having IC_{50} value 61.18 ± 1.42**,** 68.45 ± 0.33, 92.57 ±2.80 μM showed weak to good inhibitory potential. While remaining compounds are found to be almost inactive.

Molecular docking

The molecular docking program is widely used to predict the binding interaction of the compounds in the binding pocket of the enzyme. The three-dimensional (3D) crystal structure of the β-glucuronidase was retrieved from the protein databank (PDB ID: 1BHG) [Jain et al, 1996]. The B-chain of protein and hetero-atoms including cofactors were removed from the original protein data bank file. The hydrogen atoms were added to the enzyme by the 3D protonation using the MOE (Molecular Operating Environment) software (www.chemcomp.com).

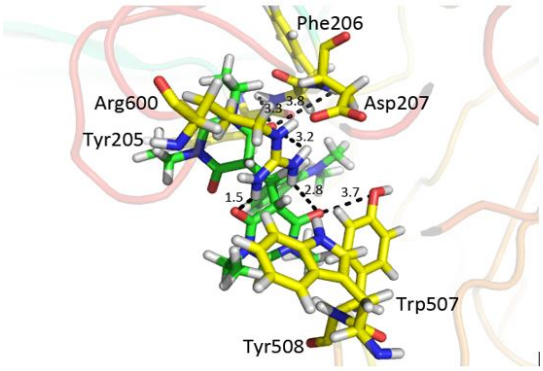


Figure 3. Docking conformation of compound **13** in the active site of β-glucuronidase

The enzyme was then energy minimized by the default parameters of the MOE for the stability and further assessment of the enzyme. The structures of the thiobarbituric acid derivative compounds (**1-15**) were built in MOE and energy minimized using the Amber99 force field and gradient: 0.05. The Site-Finder Module was utilized for prediction of the Ligand binding site in the β-glucuronidase enzyme showed that Tyr 205, Phe 206, Asp 207, Trp 507, Tyr 508 and Arg 600 were found in the binding pocket. The synthesized compounds were docked into the active site of the target enzyme in MOE by the default parameters i.e., Placement: Triangle Matcher, Rescoring: London dG. For each ligand ten conformations were generated. The top-ranked conformation of each compound was used for further analysis.

Docking studies

It was observed that all the derivatives of the thiobarbituric acid showed significant binding interactions with the active site residues of the target enzyme. From the docking conformation of the most active compound, compound **13 (IC_{50} = 29.42 ± 0.61)**, it was observed that this compound established six hydrogen bonds with active site residues and also showed good docking score (**-11.6042**) as compare to the reference compound (D-saccharic acid 1,4-lactone) having docking score of **-11.2527** and biological activity with IC_{50} of **48.4** ± **1.25**. The carbonyl oxygen atoms of the 1,3-diethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione moieties of compound **13** formed polar interactions with the Phe 206, Asp 207, Tyr 508 and Arg 600 active residues of the enzyme as shown in Figure 3**.** Tyr 205 was observed in making hydrophobic interaction with the compound. The high potency of compound **13** towards β-D-glucuronidase could be explained by these six strong hydrogen bonds.

EXPERIMENTAL

Materials and methods

^{1}HNMR spectra were recorded in DMSO-$_{d6}$ on an Avance Bruker AM 300-500 MHz instrument and TMS was used as external standard. Chemical shifts are given in δ (ppm).

Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

β-Glucuronidase Assay

The activity of β-glucuronidase was evaluated by

measuring *p*-nitro phenol absorbance at 405 nm by spectrophotometric method. Volume was 250 mL in the whole reaction. 185 mL of 0.1 M acetate buffer, test compound solution of 5 and 10 mL was the reaction mixture while incubation of enzyme solution was carried out at 37 °C for 30 min. Multi plate reader (Spectra Max plus 384) at 405 nm read the plate after the addition of 50 *m*L of 0.4 mM *p*-nitrophenyl-β-D-glucuronidase [Jain et al, 1996]. Triplicate assays were run in the whole process.

General procedure for synthesis of *bis*-thiobarbiturate derivatives (1-15)

The bis-thiobarbiturate derivatives **1-15** have been synthesized by the reaction of 1,3-diethyl-2-thioxodihydropyrimidine-4,6-(1*H*, 5*H*)-dione (*N*,*N*-diethylthiobarbituric acid, 2 mmol) with different aromatic aldehydes (1 mmol) in the presence of 5-10 mL of EtOH. Reaction mixture was stirrer in ethanol about 3 hrs. The reaction completion was monitored by periodic TLC. After completion of reaction the mixture was poured into crushed ice followed by acidification with dil. HCl. Solid precipitates was obtained, filter, dried and recrystallized from ethanol give pure product in excellent yield [Rahim et al, 2016]. The structures of all synthetic compounds 1-15 were confirmed through EI-MS and ^{1}HNMR.

Characterization of a representative analog 5,5'-((3,5-dimethoxyphenyl)methylene)bis(1,3-diethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione)

^{1}HNMR: (DMSO-d6, 400 MHz): δ 7.2 (s, 2H, H-2/6), 6.8 (s, 1H, H-4), 3.7 (m, 4H, CH_2), 3.5 (m, 4H, CH_2), 3.4 (s, 6H, OMe), 3.1 (m, 1H, CH), 1.2 (m, 12H, CH_3); EI-MS: m/z (rel. int. %): 548 (M^+, 42), 534 (45), 350 (100), 152 (34), 99 (56).

CONCLUSION

This study guided to the bioorganic and medicinal chemist that a simple one step chemistry may generate extra-ordinary bioactive compounds. During this study, we have synthesized fifteen (15) simple thiobarbituric acid derivatives and evaluated their inhibitory potential against β-glucuronidase enzyme. All compounds were identified as excellent inhibitors. This study discovered a novel class of β-glucuronidase inhibitors. The proposed scaffold of β-glucuronidase inhibitors offers the chance of expedient further modifications that could give rise to lead structures with more improved inhibitory activity and selectivity towards the enzyme.

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Affiliations and Corresponding Informations

Corresponding: Imad Uddin
Email: imad_u@yahoo.com
Phone: 03452232377

Hayat Ullah:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

Fahad Khan:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

Fazal Rahim:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

Zia Ur Rehman:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

Muhammad Taha:
Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), University of Dammam, Dammam 31441, Saudi Arabia

Abdul Wadood:
Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan-23200, Pakistan;

Fawad Ahmad:
Department of Human Genetics, Hazara University, Mansehra-21300, Khyber Pukhtunkhwa, Pakistan

Zainul Wahab:
Department of Conservation Sciences, Hazara University, Mansehra-21300, Khyber Pukhtunkhwa, Pakistan

Imad Uddin:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

Khalid Zaman:
Department of Chemistry, Hazara University Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.