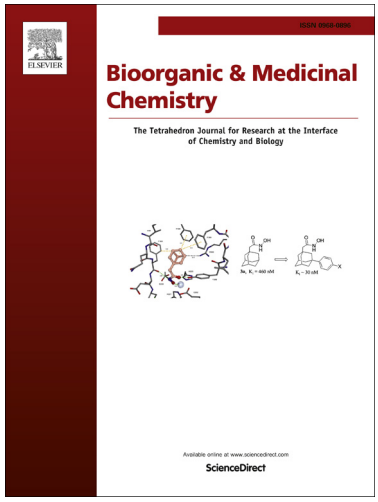
Accepted Manuscript



Synthesis and Structure-activity Relationship of Thiobarbituric Acid Derivatives as Potent Inhibitors of Urease

Khalid Mohammed Khan, Fazal Rahim, Ajmal Khan, Muhammad Shabeer, Shafqat Hussain, Wajid Rehman, Muhammad Taha, Momin Khan, Shahnaz Perveen, M. Iqbal Choudhary

PII: S0968-0896(14)00416-7
DOI: http://dx.doi.org/10.1016/j.bmc.2014.05.057
Reference: BMC 11618

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 27 April 2014
Revised Date: 22 May 2014
Accepted Date: 23 May 2014

Please cite this article as: Khan, K.M., Rahim, F., Khan, A., Shabeer, M., Hussain, S., Rehman, W., Taha, M., Khan, M., Perveen, S., Iqbal Choudhary, M., Synthesis and Structure-activity Relationship of Thiobarbituric Acid Derivatives as Potent Inhibitors of Urease, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.05.057

Synthesis and Structure-activity Relationship of Thiobarbituric Acid Derivatives as Potent Inhibitors of Urease

Khalid Mohammed Khan,[1*] Fazal Rahim,[2] Ajmal Khan,[1] Muhammad Shabeer,[2] Shafqat Hussain,[1] Wajid Rehman,[2] Muhammad Taha,[3,4] Momin Khan,[6] Shahnaz Perveen,[5] and M. Iqbal Choudhary[1]

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

[2]Depatment of Chemistry, Hazara University, Mansehra, Pakistan

[3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia, [4]Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia,

[5]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan

[6]Department of Chemistry, Abdul Wali Khan University Mardan, Mardan-23200, Pakistan.

Abstract

A series of thiobarbituric acid derivatives **1-27** were synthesized and evaluated for their urease inhibitory potential. Exciting results were obtained from the screening of these compounds **1-27**. Compounds **5**, **7**, **8**, **11**, **16**, **17**, **22**, **23** and **24** showed excellent urease inhibition with IC_{50} values 18.1 ± 0.52, 16.0 ± 0.45, 16.0 ± 0.22, 14.3 ± 0.27, 6.7 ± 0.27, 10.6 ± 0.17, 19.2 ± 0.29, 18.2 ± 0.76 and 1.61± 0.18 μM, respectively, much better than the standard urease inhibitor thiourea (IC_{50} = 21 ± 0.11 μM). Compound **3**, **4**, **10**, and **26** exhibited comparable activities to the standard with IC_{50} values 21.4 ± 1.04 and 21.5 ±

*Corresponding Author: khalid.khan@iccs.edu, hassaan2@super.net.pk, Tel.: 0092-21-34824910; Fax: 0092-21-34819018

0.61μM, 22.8 ± 0.32, 25.2 ± 0.63, respectively. However the remaining compounds also showed prominent inhibitory potential The structure-activity relationship was established for these compounds. This study identified a novel class of urease inhibitors. The structures of all compounds were confirmed through spectroscopic techniques such as EI-MS and ^{1}HNMR.

Keywords: Thiobarbituric acid analogs, Synthesis, Urease Inhibition, SAR Studies.

1. Introduction

Urease enzyme is a virulence factor in certain human and animal ailments. It contributes to the development of kidney stones, pyelonephritis, peptic ulcers leading to gastric cancers and other diseases.[1] It also causes the pathogenesis of pyelonephritis, hepatic encephalopathy, hepatic coma urolithiasis, ammonia and urinary catheter encrustation.[2] The obvious cure for treating bacterial infection with antimicrobials, however, often proved to be unsuccessful.[3] Currently it was reported that the gastric cancer [4-5] is the fourth most common cancer and the second most common cause of cancer-related deaths worldwide.[6] It is also a cause of pathologies due to *Helicobacter pylori, which* lets bacteria to persist at the low pH of the stomach during colonization lead pathogenesis of gastric and peptic ulcer which in the long run may cause cancer.[7]

The barbiturates and thiobarbiturates showed a wide range of pharmacological applications, such as general anesthesia, sedation and anticonvulsant and anxiolytic effects. Barbiturate compounds also showed urease inhibition.[8] Barbituric and thiobarbituric acid derivatives also exhibited antimicrobial, [9-10] antifungal, [11] antiviral, [12] and antitumor activities.[13] From literature study it is clear that barbiturates and thiobarbiturate derivatives show diverse biological activities such as potential mushroom tyrosinase inhibition, [14] antituberculosos, [15] radio-sensitization, [16] anticancer with anti-inflammatory activities, [17] inhibition for diaminopimelate aminotransferase, [18] and anesthesia.[19] Barbiturate analogues also showed anti-proliferative activity.[20]

Our research group is involved in the search for simple but biologically interesting molecules that are easy to synthesize with no tedious chemistry and that could be accomplished in just fewer steps with high yields. This type of chemistry is easily and ideally adopted by the pharmaceutical industry for commercialization. Previously, our

research group reported arylidene barbiturates as urease inhibitors their other activities.[21] In view of these studies; we planned to synthesize thiobarbiturate derivatives with the aim to discover their urease inhibition.

1. Results and discussion

2.1 Chemistry

The synthesis of thiobarbituric acid derivatives (**1-27**) was carried out by the reaction of thiobarbituric acid (1 mmol) with different aromatic aldehyde (1 mmol) in the presence of 10 ml of 20% NaOH. The reaction mixture was stirred for 12 h. The completion of reaction was monitored by TLC. After completion of the reaction, the mixture was poured onto crushed ice followed by acidification with dilute hydrochloric acid. The products (**1-27**) were precipitated out; the solid was filtered, dried and recrystallized from ethanol (Scheme-1, Table-1). Structures of synthetic compounds were identified by spectroscopic methods such as ^{1}H NMR and EIMS. All synthetic derivatives furnished satisfactory elemental analyses.

Insert Scheme 1 here

Insert Table 1 here

2.2 Urease Inhibitory Activity

Compounds **1**-**27** were evaluated for inhibition of urease enzyme. All compounds showed potent urease inhibitory activity (Table-2). Compounds **5**, **7**, **8**, **11**, **16**, **17**, **22**, **23** and **24** showed excellent urease inhibition with IC_{50} values 18.1 ± 0.52, 16.0 ± 0.45, 16.0 ± 0.22, 14.3 ± 0.27, 6.7 ± 0.27, 10.6 ± 0.17, 19.2 ± 0.29, 18.2 ± 0.76 and 1.61± 0.18 μM, respectively, much better than the standard inhibitor thiourea (IC_{50} = 21 ± 0.11 μM). Compound **3**, **4**, **10**, and **26** exhibited comparable activities to the standard with IC_{50} values 21.4 ± 1.04 and 21.5 ± 0.61μM, 22.8 ± 0.32, 25.2 ± 0.63, respectively . However, compounds **1**, **2**, **6**, **9**, **12**, **14**, **15**, **18**, **19**, **20**, **21**, and **25** also exhibited striking inhibitory potential with IC_{50} values 46.5 ± 0.56, 47.3 ± 0.62, 62.8 ± 1.71, 43.3 ± 1.06, 42.5 ± 1.3, 50.3 ± 0.81, 32.3 ± 0.62, 58.5 ± 1.28, 42.1 ± 1.91, 29.4 ± 0.59, 32.1 ± 0.34, and 32.7 ± 0.82 μM, respectively.

Structure-activity relationship suggested that the urease activity of a particular molecule is apparently governed by the substitution present at aromatic residues. The *p*-thiomethyl substituted analog **5** (IC_{50} = 18.1 ± 0.52 μM), pyridin-4-yl **7** (IC_{50} = 16.0 ± 0.45 μM), 2-methyl-*o*-pyridin-2-yl **8** (IC_{50} = 16.0 ± 0.22 μM), 3,4-dimetoxy **11** (IC_{50} = 14.3 ± 0.27 μM), *p*-phthalaldehydic **16** (IC_{50} = 6.7 ± 0.27 μM), 3,5-dibromo-4-hyroxy **17** (IC_{50} = 10.6 ± 0.17 μM), 2-hydroxy-3-methoxy **22** (IC_{50} = 19.2 ± 0.29 μM), 2-methylfuryl **23** (IC_{50} = 18.2 ± 0.76 μM), and 3,4-dihydroxy **24** (IC_{50} = 1.61 ± 0.18 μM), respectively, showed excellent inhibitory activities among the series. It was observed that variation in substitution pattern of benzaldehyde or aromatics resulted a difference in activities. Compound **24** (3, 4-dihydroxy analog) is found to be the most active among the series with IC_{50} value 1.61± 0.18 μM. The two hydroxyl groups on phenyl ring might be involved in hydrogen bonding with nickel atoms present in urease enzyme. The compound **16** got second position among the series with IC_{50} value 6.7± 0.27 μM. This compound has one more sulfur group which might help in coordination with the nickels. The compounds **17** and **22** having *p*-hydroxy and *o*-hydroxy group with IC_{50} values 10.6 ± 0.17 and 19.2 ± 0.29 μM, respectively, also showed potent inhibition. The slight activity difference is due to differences in position of hydroxyl groups on the phenyl ring. The *p*-pyridyl **7** and 2-methyl-*o*-pyridyl **8** have almost identical urease inhibition with IC_{50} values 16.0 ± 0.45, 16.0 ± 0.22 μM. The excellent inhibitory potential of these two compounds may be due to the lone pair interaction of pyridines nitrogens with nickels. The *p*-thiomethyl substituted **5** and 3,4-dimetoxy **11** having IC_{50} values of 18.1 + 0.52 and 14.3 + 0.27 μM, respectively, have substituents with electron donating inductive effect. The 2-methylfuryl analog **23** have IC_{50} value 18.2 ± 0.76 μM displayed greater potential that may be due to lone pair interaction of furfuryl oxygen with nickel atom. The 2-naphthol residue **3** and thiophenyl analog **4** displayed IC_{50} values 21.4 ± 1.04 and 21.5 ± 0.61 µM, respectively, were found to have comparable IC_{50} values with the standard. The 4-hydroxy-3,5-dimethoxy substituted analog **1**, 3-bromo-4,5-dimethoxy **2**, coumaryl **6**, 4-bromo-2,5-dimethoxy **9**, 2-hydroxy-4-methoxy **10**, 4-hydroxy-3-iodo-5-methoxy **12**, 3,5-dichloro-2-hydroxy **14**, 2-amino **15**, *N,N*-dimethylamino **18**, 2-methyl **19**, *p*-ethoxy **20**, 2,4-dihydroxy **21**, 2-hydroxy-5-methoxy **25** and 2,3,4-trihydroxy **26** showed good to excellent inhibition. The dimethoxy analogs i.e. 4-hydroxy-3,5-

dimethoxy substituted analog **1**, 3-bromo-4,5-dimethoxy analog **2**, and 4-bromo-2,5-dimethoxy analog **9,** having IC_{50} values 46.5 ± 0.56, 47.3 ± 0.62 and 43.3 ±1.06 μM, respectively, showed almost good potential. The change in position of substituents creates a difference of activities in smaller magnitude. The compounds having both methoxy and hydroxyl like in 2-hydroxy-4-methoxy analog **10**, 4-hydroxy-3-iodo-5-methoxy analog **12** and 2-hydroxy-5-methoxy analog **25** showed excellent inhibition. The compounds **10** and **25** having same substituents but the position of substituents are different that brings a difference in their activities. By comparing the activities of mono, di and tri hydroxyl analogs like 3, 5-dichloro-2-hydroxy **14**, 2,4-dihydroxy **21** and 2,3,4-trihydroxy **26**, we found that urease inhibitory potential increases with the increase in the number of hydroxyl groups on aromatic ring. By changing the substituent from hydroxyl to amino group such as 2-amino **15** and *N,N*-dimethylamino **18** it was observed that the unsubstituted amino analog showed greater potential than the substituted one which might be due to the obvious reason of better interaction of free nitrogen with nickel atom. Comparing the inhibitory potential of compound 2-methyl **19** and *p*-ethoxy analog **20**, we observed that as the electron donating power of a group increases, the inhibitory potential proportionally increases. The coumaryl **6,** anthranyl **13** and the indolyl **27** showed weak inhibition with IC_{50} values of 62.8 ± 1.71, 137.2±1.92 and 170.8±2.08μM, respectively. All these compounds showed weak inhibition and this might be due to the steric hindrance.

Insert table 2 here

2. 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, herein, we synthesized twenty-seven thiobarbituric acid derivatives through a simple one step chemistry and evaluated for their inhibitory potential against urease. Most of these compounds were identified as excellent urease inhibitors. This study discovered a new class of urease inhibitors. These lead compounds have opened a new avenue that has the potential to be novel inhibitors against urease through further modifications.

3. Material and methods

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

Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. CHN Analyses were carried out a Carlo Erba Strumentazion-Mod-1106, Italy. 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.

4.1 General procedure for the synthesis of compounds 1-27

The syntheses of thiobarbituric acid derivatives **(1-27)** were carried out by the reaction of thiobarbituric acid (1 mmol) with different aromatic aldehyde (1 mmol) in the presence of 10 ml of 20% NaOH. The reaction mixture was stirred for 12 h. The completion of reaction was monitored by periodic TLC. The completion of the reaction was monitored periodically by TLC. After completion of the reaction, the mixture was poured into crushed ice followed by acidification with dilute HCl. The product precipitated as a solid which was filtered, dried and recrystallized from ethanol.

4.1.1. 5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (1)

Yield: 0.88 g (93%); MP 263 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.3 (s, 2H, NH), 11.1 (s, 1H, OH), 8.3 (s, 1H, CH aldehydic), 7.3 (s, 2H, H-2/6), 3.7 (s, 6H, OCH_3); EI-MS: *m/z* (rel. int. %): 308 (M^+, 100), 280 (69), 224 (44), 190 (35), 165 (30).

4.1.2 5-(2-Bromo-4,5-dimethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (2)

Yield: 0.89 g (92%); MP 271 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 2H, NH), 8.5 (s, 1H, H-3), 8.2 (s, 1H, CH aldehydic), 7.9 (s, 1H, H-6), 3.9 (s, 6H, OCH_3); EI-MS: *m/z* (rel. int. %): 306 (M^+, 90), 304 (90) 277 (93), 260 (100), 247 (29) 179 (43).

4.1.3 5-[(2-Hydroxy-1-naphthyl)methylene]-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (3)

Yield: 0.89 g (93%); MP 255 °C:^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3(s, 1H, NH),11.2 (s, 1H, OH),7.7 (d, 2H, $J_{5,6/8,7}$ = 8.2 Hz, H-5/8), 7.5 (d, 1H, $J_{4,3}$ = 8.0 Hz, H-4), 7.3 (m, 2H, H-6/ CH aldehydic), 7.1 (d, 1H, $J_{3,4}$ = 8.4 Hz, H-3). EI-MS: *m/z* (rel. int. %): 298 (M^+, 100), 270 (56), 255 (45), 176 (60), 145 (34).

4.1.4 5-(2-Thienylmethylene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (4)

Yield: 0.89 g (93%); MP 246 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 8.4 (s, 1H, CH aldehydic), 8.1 (d, 1H, $J_{4,3}$ = 3.5 Hz, H-4), 7.7 (d, 1H, $J_{2,3}$ = 3.6 Hz, H-2), 7.5 (m, 1H,H-3); EI-MS: *m/z* (rel. int. %): 238 (M^+, 100), 220 (74), 180 (55), 159 (36) 115 (23).

4.1.5. 5-[4-(Methylsulfanyl)benzylidene]-2-thioxodihydro-4,6(1H,5H) pyrimidinedione (5)

Yield: 0.90 g (93%); MP 262 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 8.3 (s, 1H, CH aldehydic), 8.2 (d, 2H, $J_{3,2/5,6}$ = 6 Hz, H-3/5), 7.4 (d, 2H, $J_{2,3/6,5}$ = 8.4 Hz, H-2/6), 2.6 (s, 3H, SCH_3); EI-MS: *m/z* (rel. int. %): 278 (M^+, 278), 260 (74), 244 (93), 176 (66), 134 (50).

4.1.6. 5-[(6-Bromo-4-chloro-2-oxo-2H-chromen-3-yl)methylene]-2-thioxodihydro 4,6(1H,5H)-pyrimidinedione (6)

Yield: 0.89 g (93%); MP 258 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.3 (s, 1H, NH), 12.2 (s, 1H, NH), 8.5 (s, 1H, H-5), 8.4 (d, 1H, $J_{7',8'}$ = 8.2 Hz, H-7'), 8.2 (d, 1H, $J_{8,7}$ = 8.2 Hz, H-8), 8.0 (s, 1H, CH aldehydic); EI-MS: *m/z* (rel. int. %): 413 (M^+, 100), 380 (60), 320 (45), 290 (63), 250 (33).

4.1.7 5-(4-Pyridinylmethylene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (7)

Yield: 0.90 g (95%); MP 283 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 11.75 (s, 2H, NH), 8.64 (d, 2H, $J_{3,2/5,6}$ = 6.6 Hz, H-3/5), 7.7 (d, 2H, $J_{2,3/6,5}$ = 5.7 Hz, H-2/6), 6.2 (s, 1H, CH aldehydic); EI-MS: *m/z* (rel. int. %): 233 (M^+, 100), 174 (15), 135 (24), 115 (21) 103 (24).

4.1.8 5-[(6-Methyl-2-pyridinyl)methylene]-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (8)

Yield: 0.88 g (93%); MP 272 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 11.7 (s, 2H, NH), 8.3 (m, 1H, H-3) 7.7 (d, 1H, $J_{2,3}$ = 7.8Hz, H-2),7.6 (d, 1H, $J_{3,4}$ = 8.1Hz, H-4), 6.1 (s,1H, CH aldehydic), 1.5 (s, 3H, CH_3); EI-MS: *m/z* (rel. int. %): 247 (M^+, 62), 219 (69), 148 (80), 143 (100) 116 (47).

4.1.9 5-(4-Bromo-2,5-dimethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (9)

Yield: 0.96 g (92%); MP 295 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH) 8.4 (s, 1H, H-2), 8.0 (s, 1H, H-5), 7.4 (s, 1H, CH aldehydic) 3.9 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3). EI-MS: *m/z* (rel. int. %): 370 (M^+, 62), 340 (100), 338 (75), 282 (51), 280 (56).

4.1.10. 5-(3-Hydroxy-4-methoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (10)

Yield: 0.89 g (93%); MP 276 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 9.5 (s, 1H,OH), 8.2 (s, 1H, CH aldehydic), 8.1 (s, 1H, H-2), 7.78 (d, 1H, $J_{5,6}$ = 6.9 Hz, H-5), 7.72 (dd, 1H, $J_{6,5}$ = 9.2. $J_{6,2}$ = 2.1 Hz, H-6), 3.9 (s, 3H, OCH_3); EI-MS: *m/z* (rel. int. %): 278 (M^+, 100), 260 (16), 218 (14), 179 (20), 132 (15).

4.1.11. 5-(3,4-Dimethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (11)

Yield: 0.96 g (92%); MP 268 °C: ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 8.4 (d, 1H, $J_{2,6}$ = 1.6Hz, H-2), 8.3 (s, 1H, CH aldehydic), 7.9 (m, 1H, H-6), 7.13 (d, 1H, $J_{5,6}$ = 8.8 Hz, H-5), 3.9 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3); EI-MS: *m/z* (rel. int. %): 292 (M^+, 100), 277 (34), 261 (61), 232 (18), 202 (40).

4.1.12. 5-(4-Hydroxy-3-iodo-5-methoxybenzylidene)-2-thioxodihydro-4,6(1H,5H) pyrimidinedione(12)

Yield: 0.91 g (90%); MP 300 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.3 (s, 2H, NH), 8.6 (s, 1H, CH aldehydic), 8.3 (s, 1H, H-2), 8.2 (s, 1H, H-6), 3.9 (s, 3H, OCH_3); EI-MS: *m/z* (rel. int. %): 404 (M^+, 100), 402 (18), 305 (15), 277 (10) 218 (25).

4.1.13. 5-(1,4-Dihydro-9-anthracenylmethylene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (13)

Yield: 0.91 g (88%); MP 285 °C: ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ 12.6 (s, 1H, NH), 12.2 (s, 1H, NH), 8.9 (s, 1H, H-6), 8.7 (s, 1H, CH aldehydic), 8.2 (d, 2H, $J_{2,3/10,9}$ = 8.4 Hz, H-2/10), 7.9 (d, 2H, $J_{7,8/5,4}$ = 8.4 Hz,H-7/5), 7.6 (m, 4H, H-3/4/8/9); EI-MS: *m/z* (rel. int. %): 334 (M^+, 7), 331 (73), 230 (100), 202 (95), 201 (41).

4.1.14. 5-(3,5-Dichloro-2-hydroxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (14)

Yield: 0.88 g (92%); MP 281 °C: ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ 12.4 (s, 2H, NH), 11.6 (s, 1H, OH), 7.5 (s, 1H, H-4), 7.09 (s, 1H, H-6), 5.2 (s, 1H, CH aldehydic); EI-MS: *m/z* (rel. int. %): 317 (M^+, 100), 300 (82), 240 (25), 211 (54), 115 (56).

4.1.15. 5-(2-Aminobenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (15)

Yield: 0.89 g (93%); MP 249 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.6 (s, 2H, NH), 8.5 (s, 1H, CH aldehydic), 8.2 (d, 1H, $J_{3,4}$ = 8.1 Hz, H-3), 7.9 (d, 1H, $J_{6,5}$ = 3.6 Hz, H-6),7.6 (m, 2H, H-4/5), 4.1 (s, 2H, NH_2); EI-MS: *m/z* (rel. int. %): 247 (M^+, 44), 299 (46), 172 (100), 143 (52) 116 (46).

4.1.16. 5-(4-{[4,6-Dioxo-2-thioxotetrahydro-5(2H)pyrimidinylidene]methyl}-benzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (16)

Yield: 0.87 g (91%); MP 284 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 4H, NH), 8.2 (s, 2H, H- CH aldehydic), 7.9 (d, 2H, $J_{2,3/6,5}$ = 8.4 Hz, H-2/6), 7.8 (d, 2H, $J_{3,2/5,6}$ = 8.4 Hz, H-3/5); EI-MS: *m/z* (rel. int. %): 386 (M^+, 100), 350 (14), 314 (20), 270 (63) 220 (11).

4.1.17. 5-(3,5-Dibromo-4-hydroxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (17)

Yield: 0.85 g (89%); MP 290 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.7 (s, 1H, NH), 12.1 (s, 1H, NH), 8.7 (s, 2H,H-2/6), 8.00 (s, 1H, CH aldehydic); EI-MS: *m/z* (rel. int. %): 406 (M^+, 100), 402 (50), 327 (16), 307 (43), 276 (18), 87 (34).

4.1.18. 5-[4(Dimethylamino) benzylidene]-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (18)

Yield: 0.87 g (91%); MP 259 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 2H, NH), 8.5 (s, 1H, CH aldehydic), 8.2 (d, 2H, $J_{2,3/6,5}$ = 8.1 Hz, H-2/6), 7.9 (d, 2H, $J_{3,2/5,6}$ = 8.2 Hz, H-3/5), 3.6 (s, 6H, NMe_2); EI-MS: *m/z* (rel. int. %): 275 (M^+, 100), 273 (30), 177 (29), 172 (15) 144 (16).

4.1.19. 5-(2-Methylbenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (19)

Yield: 0.89 g (93%); MP 241 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 2H, NH), 8.9 (s, 1H, H-7), 7.9 (d, 2H, $J_{3,4/6,5}$ = 8.7 Hz, H-3/6), 7.1 (m, 2H, H-4/5),2.4 (s, 3H, CH_3); EI-MS: *m/z* (rel. int. %): 246 (M^+, 100), 203 (14), 188 (20), 159 (63), 103 (11).

4.1.20. 5-(4-Ethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (20)

Yield: 0.93 g (91%); MP 263 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.1 (s, 1H, NH), 12.0 (s, 1H, NH), 8.5(d, 2H, $J_{2,3/6,5}$ = 9.3Hz, H-2/6), 8.2 (s, 1H, CH aldehydic), 6.9 (d, 2H, $J_{3,2/5,6}$ = 9 Hz, H-3/5), 4.1 (m, 2H, OCH_2), 3.2 (m, 3H, OCH_2CH_3); EI-MS: *m/z* (rel. int. %): 276 (M^+, 100), 247 (46), 230 (29), 150 (27) 145 (16).

4.1.21. 5-(2,4-Dihydroxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (21)

Yield: 0.89 g (93%); MP 256 °C: ^{1}HNMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 11.2 (s, 1H, OH), 8.5 (d, 1H, $J_{6,5}$ = 3.6 Hz, H-6), 8.2 (s, 1H, CH aldehydic), 7.97 (s, 1H, H-3), 6.7 (d, 1H, $J_{5,6}$ = 3.6 HZ, H-5); EI-MS: *m/z* (rel. int. %): 264 (M^+, 100), 243 (10), 208 (40), 203 (100) 118 (49).

4.1.22. 5-(2-Hydroxy-3-methoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (22)

Yield: 0.90 g (95%); MP 257 °C: ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH),9.9 (s, 1H,OH), 8.7 (s, 1H, CH aldehydic), 7.8 (d, 1H, $J_{6,5}$ = 8.4 Hz, H-6), 7.2 (d, 1H, $J_{4,3}$ = 7.6 Hz,H-4), 6.8 (m, 1H, H-5), 3.8 (s, 3H, OCH_3); EI-MS: *m/z* (rel. int. %): 278 (M^+, 41), 218 (9), 208 (15), 202 (100), 119 (8).

4.1.23. 5-[(5-Methyl-2-furyl)methylene]-2-thioxodihydro-4,6(1H,5H)pyrimidinedione (23)

Yield: 0.89 g (93%); MP 235 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 12.3 (s, 1H, NH), 8.5 (d, 1H, $J_{2,3}$ = 3.6 Hz, H-2), 7.9 (s, 1H, CH aldehydic), 6.7 (d, 1H, $J_{3,2}$ = 3.6 Hz, H-3), 1.8 (s, 3H, CH_3); EI-MS: *m/z* (rel. int. %): 236 (M^+, 100), 221 (63), 176 (47) 134 (35).

4.1.24. 5-(3,4-Dihydroxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (24)

Yield: 0.96 g (94%); MP 253 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 12.3 (s, 2H, NH), 10.6 (s, 1H, OH), 9.6 (s, 1H,OH), 8.3 (s, 1H, H-2), 8.1 (s, 1H, CH aldehydic), 7.7 (d, 1H, $J_{6,5}$ = 7.2 Hz, H-6), 6.9 (d, 1H, $J_{5,6}$ = 8.4 Hz, H-5); EI-MS: *m/z* (rel. int. %): 264 (M^+, 100), 263 (85), 247 (59), 204 (32), 188 (23).

4.1.25. 5-(2-Hydroxy-5-methoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (25)

Yield: 0.89 g (93%); MP 249 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 2H, NH), 11.5 (s, 1H, OH),8.2 (s, 1H, CH aldehydic), 7.9 (d, 1H, $J_{3,4}$ = 8.2 Hz, H-6), 7.7 (s, 1H, H-6), 7.5 (d, 1H, $J_{4,3}$ = 8.4 Hz, H-5), 3.8 (s, 3H, OCH_3); EI-MS: *m/z* (rel. int. %): 278 (M^+, 90), 243 (10), 208 (40), 203 (100), 118 (49).

4.1.26. 2-Thioxo-5-(2,3,4-trihydroxybenzylidene)dihydro-4,6(1H,5H)-pyrimidinedione (26)

Yield: 0.91 g (93%); MP 255 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 2H, NH), 11.1 (s, 3H, OH), 8.8 (s, 1H, CH aldehydic), 7.5 (d, 1H, $J_{6,5}$ = 8.7 Hz, H-6), 7.07 (d, 1H, $J_{5,6}$ = 8.4 Hz, H-2); EI-MS: *m/z* (rel. int. %): 280 (M^+, 100), 260 (74), 234 (93), 176 (66), 134 (43).

4.1.27. 5-(1H-Indol-3-ylmethylene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (27)

Yield: 0.92 g (89%); MP 265 °C: ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.9 (s, 1H, N$^{'}$H), 12.2 (s, 1H, NH), 12.17 (s, 1H, NH),9.6 (s, 1H, H-2), 8.7 (s, 1H, CH aldehydic), 7.9 (d, 1H, $J_{4,5}$ = 6.6 Hz, H-4), 7.6 (d, 1H, $J_{7,6}$ = 5.7 Hz, H-7), (m, 2H, H-5/6); EI-MS: *m/z* (rel. int. %): 271 (M^+, 100), 270 (28), 196 (14), 172 (37), 168 (17).

4.2 Urease Inhibition Assay

Reaction mixtures comprising one unit of urease enzyme (*Bacillus pasteurii*) solution and 55 μL of buffers containing 100 mM urea were incubated with 5μL of test compounds (1mM concentration) at 30 $^{\circ}$C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol's method.[22] Momentarily, 45 μL each of phenol reagent and 70 (L of alkali reagent were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a micro-plate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using the Soft-Max Pro s4.5.Software (Molecular Devices, USA).

Acknowledgements: The authors are thankful to the Organization for Prohibition of Chemical Weapons (OPCW), The Netherlands (Project No. L/ ICA/ ICB/ 173681/ 12) for financial support.

References

[1] Mobley, H.; Island, M. D.; Hausinger, R. P. *Microbiol. Rev*. **1995**, *59*, 451-480.

[2] Bayerdörffer, E.; Ottenjann, R. *J. Gastroenterol*. **1988**, *23*, 93-100.

[3] Devesa, S. S.; Blot, W. J.; Fraumeni, J. F. *Changing patterns in the incidence of esophageal and gastric carcinoma in the United States, Cancer.* **1998**, *83*, 2049-2053.

[4] Howson, C. P.; Hiyama, T.; Wynder, E. L. *Epidemiol. Rev.* **1986**, *8*, 1-27.

[5] Parkin, D. M.; Bray, F. I.; Devesa, S. S. *Eur. J. Cancer*. **2001**, *37*, S4-S66.

[6] López-Muñoz, F.; Ucha-Udabe, R.; Alamo, C. *Neuropsychiatr. Dis. Treat.* **2005**, *1*, 329-343.

[7] Mobley, H.; Hausinger, R. *Microbiol. Rev.* **1989**, *53*, 85-108.

[8] Rauf, A.; Ahmed, F.; Qureshi, A. M.; Khan, A.; Qadir, M. I.; Choudhary, M. I.; Chohan, Z. H.; Youssoufid, M. H.; Haddad, T. B. *J. Chin. Chem. Soc*. **2011**, *58*, 528-537.

[9]. Dabholkar, V. V.; Ravi, T. D. *J. Serbian Chem. Soc*. **2010**, *75*, 1033-1040.

[10]. Kidwai, M.; Thakur, R.; Mohan, R. *Acta. Chim. Slov*. **2005**, *52*, 88-92.

[11]. Lee, J. H.; Lee, S.; Park, M.Y.; Myung, H. *J. Virol*. **2011**, *8*, 18. doi: 10.1186/1743-422X-8-18

[12]. Balas, V. I.; Verginadis, I. I.; Geromichalos, G. D.; Kourkoumelis, N.; Male, L.; Hursthouse, M. B.; Repana, K. H.; Yiannaki, E.; Charalabopoulos, K.; Bakas, T.; Hadjikakou, S. K. *Eur. J. Med. Chem.* **2011**, *46*, 2835-2844.

[13]. Brewer, A. D.; Minatelli, J. A.; Plowman, J.; Paull, K. D.; Narayanan, *Biochem. Pharmacol.* **1985**, *34*, 2047-2050.

[14]. Yan, Q.; Cao, R.; Yi, W.; Yu, L.; Chen, Z.; Ma, L.; Song, H. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 4055-4058.

[15]. Vijaya Laxmi, S.; Thirupathi Reddy, Y.; Suresh Kuarm, B.; Narsimha Reddy, P.; Crooks, P. A.; Rajitha, B. *Bioorganic & Med. Chem. Letters,* **2011**, *21*, 4329-4331.

[16]. Thirupathi Reddy, Y.; Sekhar, K. R.; Nidhish Sasi, P.; Narsimha Reddy.; Freeman, M. L.; Crooks, P. A. *Bioorganic & Med. Chem. Letters*. **2010**, *20*, 600-602

[17]. Narsimha Reddy Penthala, Purushothama Rao Ponugoti, Vinod Kasam, Peter A. Crooks. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1442-1446.

[18]. Chenguang, F.; Clay, M. D.; Deyholos, M. K.; John, C. *Bioorg. Med. Chem.* **2010**, *18*, 2141-2151

[19]. Orhan, D. D.; Kupeli, E.; Yesilada, E.; Ergun, F. *J. Biosci.* **2006**, *61*, 26-30.

[20]. Madadi, N. R.; Penthala, N. R.; Janganati, V.; Peter, A. Crooks. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 601-603.

[21]. a) Khan, K. M.; Ali, M.; Wadood, A.; Khan, M.; Lodhi, M. A.; Perveen, S.; Choudhary, M. I.; Voelter, W. *J. Mol. Graph. Model.* **2011**, *30*, 153-156. b) Khan, K. M.; Ali, M.; Ahmad, A.; Amyn, A.; Karim, A.; Khan, M.; Perveen, S. *J. Chem. Soc. Pak.* **2013**, *35*, 890-893. c) Khan, K. M.; Khan, M.; Karim, A.; Taha, M.; Ambreen, N.; Gojayev, A.; Perveen, S.; Choudhary, M. I. *J. Chem. Soc. Pak.* **2013**, *35*, 495-498. d) Khan, K. M.; Khan, M.; Ali, M.; Taha, M.; Hameed, A.; Ali, S.; Perveen, S.; Choudhary, M. I. *Med. Chem.* **2011**, *7*, 231-236. e) Khan, K. M.; Ali, M.; Ajaz, A.; Perveen, S.; Choudhary, M. I.; Atta-ur-Rahman *Lett. Drug Des. Discov.* **2008**, *5*, 286-291. f) **Khan**, **K. M.;** Ali, M.; Khan, M.; Taha, M.; Perveen, S. *Lett, Org. Chem.* **2011**, *8*, 28-32. g) **Khan**, **K. M.;** Ali, M.; Farooqui, T. A.; Khan, M.; Taha, M.; Perveen, S. *J. Chem. Soc. Pak.* **2009**, *31*, 823-828.

[22]. Weatherburn, M. *Anal. Chem.* **1967**, *39*, 971-974

Scheme and Tables captions

Scheme; General Scheme for the synthesis of thiobarbituric acid derivatives **1-27**

Table-1; Synthesis of thiobarbituric acid derivatives **1-27**

Table-2; Urease activity of thiobabituric acid derivatives **1-27**

Scheme 1

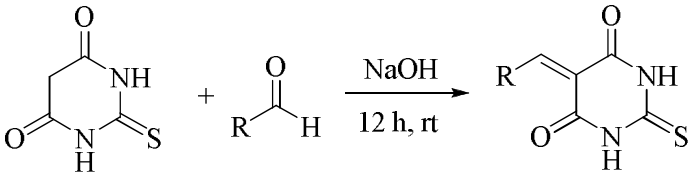


Table-1:

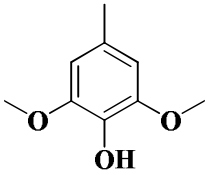
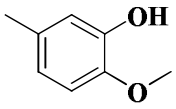
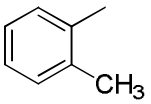
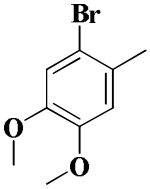
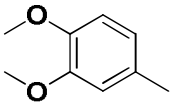
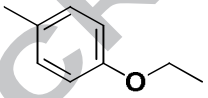
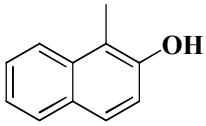
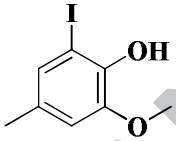
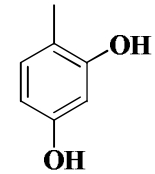
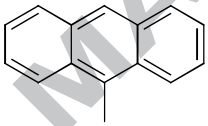
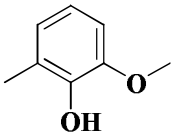
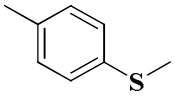
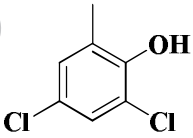
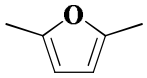
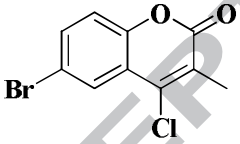
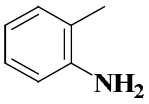
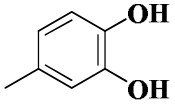
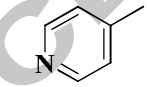
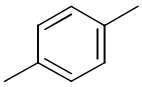
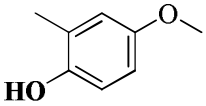
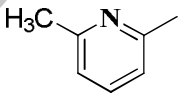
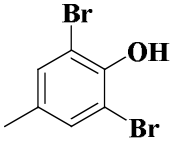
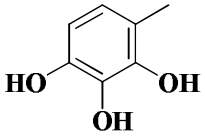
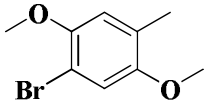
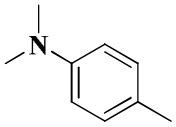
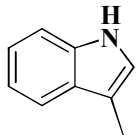
S. No.	R	Yield	S. No.	R	Yield	S. No.	R	Yield
1		93%	10		93%	19		93%
2		92%	11		92%	20		91%
3		93%	12		90%	21		93%
4		93%	13		88%	22		95%
5		93%	14		92%	23		93%
6		93%	15		93%	24		94%
7		95%	16		91%	25		93%
8		93%	17		89%	26		93%
9		92%	18		91%	27		89%

Table-2:

Compound No.	IC_{50} ± SEM[a](μ/M)	Compound No.	IC_{50} ± SEM[a] (μ/M)
1	46.5 ± 0.56	**14**	50.3 ± 0.81
2	47.3 ± 0.62	**15**	32.3 ± 0.62
3	21.4 ± 1.04	**16**	6.7 ± 0.27
4	21.5 ± 0.61	**17**	10.6 ± 0.17
5	18.1 ± 0.52	**18**	58.5 ± 1.28
6	62.8 ± 1.71	**19**	42.1 ± 1.91
7	16.0 ± 0.45	**20**	29.4 ± 0.59
8	16.0 ± 0.22	**21**	32.1 ± 0.34
9	43.3 ± 1.06	**22**	19.2 ± 0.29
10	22.8 ± 0.32	**23**	18.2 ± 0.76
11	14.3 ± 0.27	**24**	1.61 ± 0.18
12	42.5 ± 1.3	**25**	32.7 ± 0.82
13	137.2 ± 1.92	**26**	25.2 ± 0.63
Thiourea[b]	21 ± 0.11	**27**	170.8 ± 2.08

SEM[a] is the standard error of the mean, Thiourea[b] standard inhibitor for antiurease activity

Synthesis and SAR Studies of Thiobarbituric Acid Derivatives as Potent Inhibitors of Urease

Khalid Mohammed Khan,[1*] Fazal Rahim,[2] Ajmal Khan,[1] Muhammad Shabeer,[2] Shafqat Hussain,[1] Wajid Rehman,[2] Muhammad Taha,[3,4] Momin Khan,[6] Shahnaz Perveen,[5] and M. Iqbal Choudhary[1]

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

[2]Depatment of Chemistry, Hazara University, Mansehra, Pakistan

[3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia,

[4]Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia,

[5]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan

[6]Department of Chemistry, Abdul Wali Khan University Mardan, Mardan-23200, Pakistan.

*Corresponding Author: khalid.khan@iccs.edu, hassaan2@super.net.pk, Tel.: 0092-21-34824910; Fax: 0092-21-34819018

A series of thiobarbituric acid derivatives **1-27** were synthesized and evaluated for their urease inhibitory potential. Amazing results were obtained from the analysis of these compounds **1-27**. Compounds **5**, **7**, **8**, **11**, **16**, **17**, **22**, **23** and **24** showed excellent urease inhibition with IC_{50} values of 18.1 ± 0.52, 16.0 ± 0.45, 16.0 ± 0.22, 14.3 ± 0.27, 6.7 ± 0.27, 10.6 ± 0.17, 19.2 ± 0.29, 18.2 ± 0.76 and 1.61 ± 0.18 μM respectively more potent than the standard inhibitor thiourea having IC_{50} value of 21.8 ± 0.11 μM. Two compounds **3** and **4** exhibit potent inhibition with IC_{50} value 21.4 ± 1.04 and 21.5 ± 0.61μM almost same to the standard inhibitor. The rest of the compounds also showed good urease inhibition. The structure activity relationship was established for these compounds.