

Synthesis and Application of Thiobarbituric Acid Derivatives as Antifungal Agents

Rathee P¹, Tonk RK², Dalal A^{3*}, Ruhil MK⁴ and Kumar A⁵

¹Shri Baba Mastnath Institute of Pharmaceutical Sciences and Research, Rohtak-124001, India

²Delhi Pharmaceutical Sciences and Research University, New Delhi- 110017, India

³Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, India

⁴Pt. B.D Sharma University of Health Sciences, Rohtak-124001, India

⁵CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India

Abstract

Barbiturates are well known for their hypnotic effect and various methods have been worked out for their synthesis. Numerous barbiturate derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as antimicrobial, anti-mycobacterial, antifungal, anticonvulsant, analgesic, antiviral, antidepressant and anticancer activities. They also possess Xanthine oxidase (XO) inhibitory activity along with inhibitory action against protein tyrosine phosphates (PTP) 1B. 2,4,6-Trioxohexahydropyrimidine seems to be the most frequently studied barbituric type compounds and a large no. of synthetic methods for their preparation have been described in the chemistry literature. In the present work, nine new substituted TBA derivatives were synthesized by Knoevenagel condensation of indole-3-carboxaldehyde, substituted pyrazole carboxaldehyde and 3,4,5-trimethoxy carboxaldehyde with substituted thiobarbituric acid. The synthetic route for the final compounds starts with the formation of substituted biphenyl thiourea from reaction of carbon disulfide and substituted aniline in ethanol. Then the cyclization of substituted biphenyl thiourea in the presence of malonic acid and acetyl chloride afforded substituted biphenyl thiobarbituric acid. The subsequent condensation of substituted TBA with different carboxaldehyde derivatives yielded final compounds. All the final compounds were characterized by IR, ¹H NMR spectroscopy. They were screened for their antifungal activity against *C. albicans*, *A. niger* and *P. citrinum*. Newly synthesized compounds were evaluated for antifungal activity by cup plate method using Fluconazole as standard. The unsubstituted biphenyl thiobarbituric acid derivatives were found more active than the substituted biphenyl thiobarbituric acid derivatives.

Keywords: Antifungal activity; Barbiturate; Fluconazole; Thiobarbituric acid derivatives; Xanthine oxidase

Abbreviations: *A. niger*: *Aspergillus niger*; *C. albicans*: *Candida albicans*; *P. citrinum*: *Penicillium citrinum*; IR Spectroscopy: Infrared Spectroscopy; ¹H-NMR: Proton Nuclear Magnetic Resonance; TBA: Thiobarbituric Acid; XO: Xanthine Oxidase

Introduction

Among heterocyclic compounds, pyrimidine plays an essential role in chemistry and biological systems. Barbituric acids (2, 4, 6-trioxohexahydropyrimidine) is one of the most interesting derivatives of pyrimidines [1-3]. Owing to various pharmaceutical activities of 2, 4, 6-trioxohexahydropyrimidine and its derivatives have been used extensively in medicine and biological researches. Barbituric acid itself has been used as a reactant to form a large class of barbiturate drugs which are used as hypnotics, sedatives, anticonvulsants, anesthetics and as CNS depressants. Due to the applications of barbiturates, exploration of new routes for the synthesis of these compounds is axiomatic [4,5] Barbiturates are the substituted derivatives of barbituric acid (malonyl urea). One of the ways of making potentially biologically active compound is modification on C-5 of barbituric acid. Combination of barbituric acid moiety with other pharmacophoric groups gives possibility to synthesize numerous derivatives with potential biological effects [6].

One of the major problems with barbituric acids in medicinal chemistry is the frequent lack of lipid solubility preventing them from penetrating the blood-brain barrier. The usual method for increasing fat solubility in such groups usually results in the desired solubility in lipids; it also modifies the steric configuration of the molecule considerably and may interfere with the ability of the modified drug

to attach itself to the receptor sites on which its action is exerted [7]. Another method of increasing lipid solubility is the replacement of oxygen of barbituric acid at 2-position by sulfur which is known as 2-thiobarbituric acid (1, 2, 3, 4, 5, 6-hexahydro-4, 6-dioxo-2-thioxo pyrimidine). TBA differs from barbituric acid (BA) only in the presence of a sulfur atom instead of oxygen atom at the number 2 carbon. It is the parent compound of a class of drugs, the thiobarbiturates, which are analogous in their effects to barbiturates [8]. Positioning of sulfur atom to replace carbonyl oxygen at the C-2 position incurs higher lipid solubility [9]. This substitution results in higher fat solubility, short duration of action and rapid onset of activity [10]. The antifungal activity appears to be highly dependent on the lipophilic character as measured by the 1-octanol/water partition coefficient [11].

Active methylene compounds show reactions characteristic of the functional groups attached to the methylene group. They also show reactions because of the considerably acidic hydrogen in the active methylene group [12,13]. Knoevenagel condensation is a reaction between an active methylene compound and a carbonyl compound in which there is nucleophilic addition of the active methylene compound

***Corresponding author:** Dalal A, Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, India, E-mail: ruhalanita@gmail.com

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to the carbonyl group followed by dehydration. Malonic ester synthesis is a reaction where an ester of malonic acid is alkylated at the carbon alpha to both carbonyl groups, and then converted to a substituted acetic acid. TBA derivatives have been reported to possess a broad spectrum of biological activities namely antifungal, antimicrobial, and anti-tubercular, herbicides, antioxidants, antiviral & anticonvulsant activities [14-17]. Due to their wide range of biological activity thiobarbituric ring constitutes a relevant synthetic target in pharmaceutical industry [18].

Materials and Methods

The chemicals used for the experimental work were commercially procured from various chemical units: Haryana Scientific Engineering Corporation Ltd., E. Merck India Ltd. Melting points were determined by open tube capillary method and were uncorrected. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in benzene-acetone (9:1), benzene-acetone (8:2), benzene-acetone (6:4) solvent systems; the spots were located under iodine vapors and UV light. The IR spectra were recorded by using BIO-RAD FT-IR spectrometer by making KBr pellets. ¹H-NMR spectra were recorded on Bruker 300 MHz and 400 MHz instrument in solvent (DMSO-*d*₆, CDCl₃). The chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard.

Common method of synthesis of (1, 2, 3, 4, 5, 6-Hexa Hydro-4, 6-Dioxo- 2- Thioxopyrimidine) (Scheme 1(3))

A mixture of aniline (20 g, 19.6 mL, and 0.215 mol) and carbon disulfide (25 g, 19.7 mL, 0.329 mole) in absolute alcohol (50 mL) was refluxed on water bath for 12 hr or until the reaction mixture solidified. The separated product filtered and washed with excess of dilute HCL (1:1) to remove unreacted aniline and finally with cold alcohol. The 1, 3- diphenylthiourea thus obtained is crystallized from ethanol as white shining crystals [19]. A mixture of 1, 3-diphenylthiourea (2.28g, 0.01mol), dry malonic acid (1.1 g, 0.01 mol) and acetyl chloride (10 mL) was stirred at 40-50°C (oil bath) for 4-6 hrs. The progress of the reaction was followed by TLC. The reaction was stopped when all the thiourea has been consumed. The mixture was stirred with crushed ice and the separated product is filtered, washed with water and crystallized with acetic acid [20].

General procedure for the synthesis of carboxaldehyde derivatives of substituted thiobarbituric acids (Scheme 1(4-12)).

5-(Indol-3-yl) barbituric acid was prepared by Knoevenagel condensation of indol-3-carboxaldehyde and barbituric acid in ethanol using piperidine as a base. Barbituric acid (5.5 g, 0.039 mol) and indole-3-carboxaldehyde (5.66 g, 0.039 mol) in ethanol (75 mL) was heated under reflux for 15 minutes. Piperidine (1 mL) was added in one portion and the reflux was continued for further 5-10 hrs. The reaction mixture was cooled to room temperature and the solid formed was filtered, washed with ethanol (2 × 20 mL) and dried 5-(Indol-3-yl) barbituric acid was recrystallized from ethanol as dark yellow powder [21].

5-(1H-Indol-3-yl-methylidene)-1, 3-diphenyl-2-thioxo dihydropyrimidine-4, 6(1H, 5H)-dione (Scheme 1(4)).

Pale yellow; IR (KBr) cm⁻¹: 3244 (NH str.), 2923, 2835 (C-H), 1656 (C=O), 1522 (C=C), 1275 (C-N), 1039 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.13-7.21 (m, 4H, H_{indole}), 7.39-7.57 (m, 10H, H_{phenyl}), 7.82 (s, 1H, C₂-H_{indole}), 8.51 (s, 1H, =CH-), 11.23 (s, 1H, NH_{indole}). C, H, N % analysis calculated: C, 70.90; H, 4.05; N, 9.92; Found: C, 70.96; H, 4.08; N, 9.88.

5-(1H-Indol-3-yl-methylidene)-1, 3-di-o-tolyl-2-thioxo dihydropyrimidine-4, 6(1H, 5H)-dione (Scheme 1(5)).

Lemon yellow; IR (KBr) cm⁻¹: 3256 (NH str.), 2914, 2839 (C-H), 1662 (C=O), 1519 (C=C), 1278 (C-N), 1044 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.38 (s, 6H, 2×CH₃), 7.15-7.24 (m, 4H, H_{indole}), 7.34-7.50 (m, 8H, H_{phenyl}), 7.79 (s, 1H, C₂-H_{indole}), 8.53 (s, 1H, =CH-), 11.17 (s, 1H, NH_{indole}).

5-(1H-Indol-3-yl-methylidene)-1, 3-di-p-tolyl-2-thioxo dihydropyrimidine-4, 6(1H, 5H)-dione (Scheme 1(6)).

Pale yellow; IR (KBr) cm⁻¹: 3252 (NH str.), 2919, 2833 (C-H), 1667 (C=O), 1522 (C=C), 1270 (C-N), 1049 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.34 (s, 6H, 2×CH₃), 7.17-7.26 (m, 4H, H_{indole}), 7.31-7.47 (m, 8H, H_{phenyl}), 7.72 (s, 1H, C₂-H_{indole}), 8.48 (s, 1H, =CH-), 11.24 (s, 1H, NH_{indole}).

5-[(5-chloro-3-methyl-1H-pyrazol-4-yl) methylidene]-1, 3-diphenyl-2-thioxodihydro pyrimidine-4, 6(1H, 5H)-dione (Scheme 1(7)).

Dark yellow; IR (KBr) cm⁻¹: 3258 (NH str.), 2932, 2839 (C-H), 1664 (C=O), 1526 (C=C), 1270 (C-N), 1043 (C=S), 736 (C-Cl). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.31 (s, 3H, CH₃), 7.41-7.59 (m, 10H, H_{phenyl}), 8.45 (s, 1H, =CH-), 11.23 (s, 1H, NH_{pyrazole}). C, H, N % analysis calculated: C, 59.64; H, 3.58; N, 13.25; Found: C, 59.58; H, 3.57; N, 13.23.

5-[(5-chloro-3-methyl-1H-pyrazol-4-yl) methylidene]-1, 3-bis (2-methylphenyl)-2-thioxodihydro pyrimidine-4, 6(1H, 5H)-dione (Scheme 1(8)).

Reddish yellow; IR (KBr) cm⁻¹: 3258 (NH str.), 2937, 2841 (C-H), 1669 (C=O), 1532 (C=C), 1268 (C-N), 1037 (C=S), 732 (C-Cl). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.31 (s, 3H, CH₃), 2.36 (s, 6H, 2×CH₃), 7.32-7.54 (m, 8H, H_{phenyl}), 8.39 (s, 1H, =CH-), 11.26 (s, 1H, NH_{pyrazole}).

5-[(5-chloro-3-methyl-1H-pyrazol-4-yl) methylidene]-1, 3-bis (4-methylphenyl)-2-thioxodihydro pyrimidine-4, 6(1H, 5H)-dione (Scheme 1(9)).

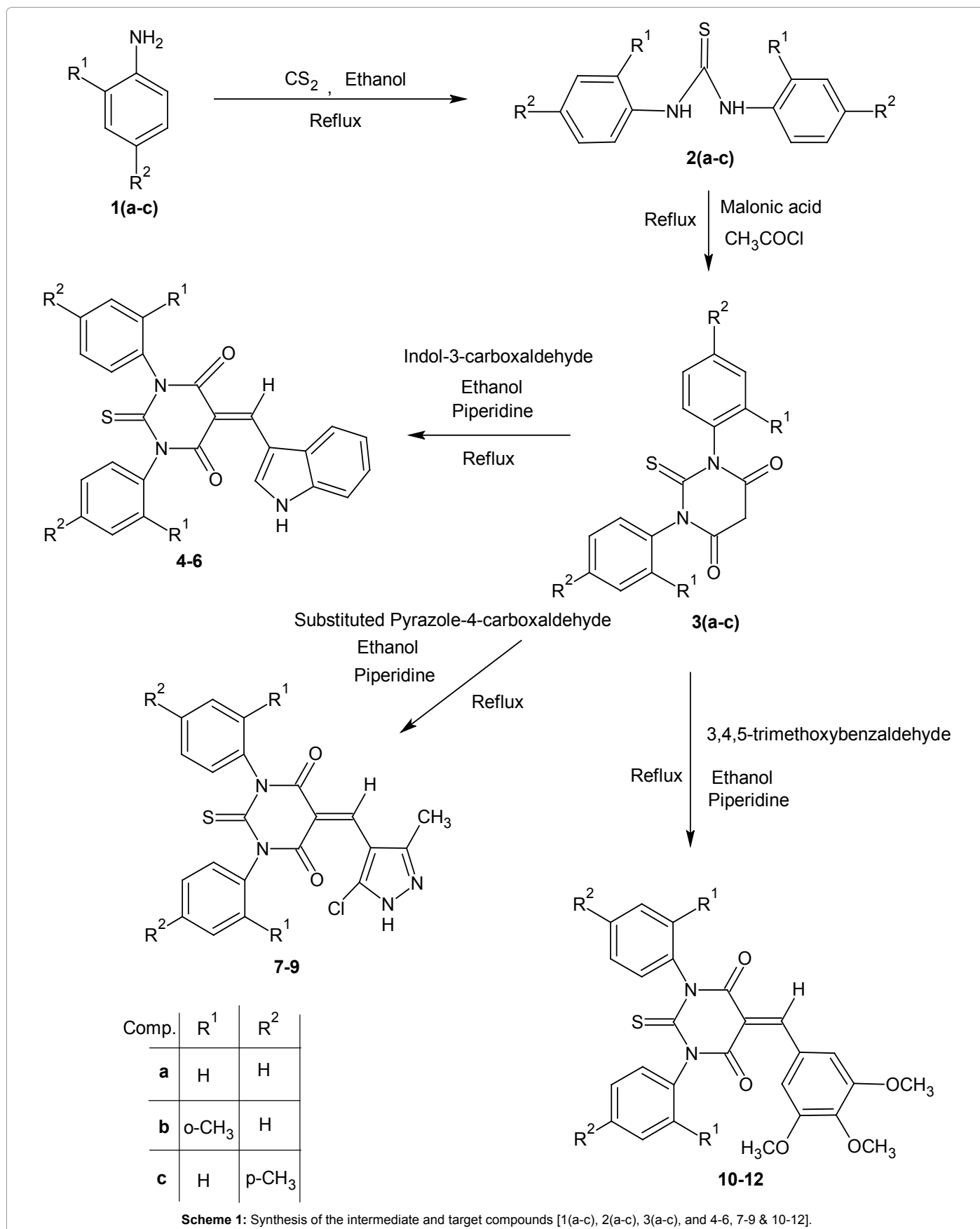
Creamish yellow; IR (KBr) cm⁻¹: 3244 (NH str.), 2933, 2846 (C-H), 1666 (C=O), 1529 (C=C), 1262 (C-N), 1033 (C=S), 746 (C-Cl). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.29 (s, 3H, CH₃), 2.41 (s, 6H, 2×CH₃), 7.36-7.58 (m, 8H, H_{phenyl}), 8.34 (s, 1H, =CH-), 11.20 (s, 1H, NH_{pyrazole}).

5-(3,4,5-trimethoxybenzylidene)-1,3-diphenyl-2-thioxo-dihydropyrimidine-4,6(1H, 5H)-dione (Scheme 1(10)).

Pale yellow; IR (KBr) cm⁻¹: 3244 (NH str.), 2923, 2834 (C-H), 1660 (C=O), 1529 (C=C), 1263 (C-N), 1049 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 3.42 (s, 9H, 3×OCH₃), 7.19-7.23 (m, 2H, H_{methoxyphenyl}), 7.36-7.55 (m, 10H, H_{phenyl}), 8.38 (s, 1H, =CH-). C, H, N % analysis calculated: C, 65.81; H, 4.67; N, 5.90; Found: C, 65.87; H, 4.68; N, 5.88.

5-(3,4,5-trimethoxybenzylidene)-2-thioxo-1,3-di-o-tolyl-dihydropyrimidine-4,6(1H, 5H)-dione (Scheme 1(11)).

Lemon yellow; IR (KBr) cm⁻¹: 3239 (NH str.), 2916, 2832 (C-H), 1667 (C=O), 1534 (C=C), 1258 (C-N), 1042 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 3.48 (s, 9H, 3×OCH₃), 7.14-7.19 (m, 2H, H_{methoxyphenyl}), 7.33-7.51 (m, 8H, H_{phenyl}), 8.41 (s, 1H, =CH-).



Compound No.	R ¹	R ²	Time of reflux (h)	Yield (%)	M. P. (°C)	M. Formula	R _f Value
4	-H	-H	6	60	150-152	C ₂₅ H ₁₇ N ₃ O ₂ S	0.68
5	-CH ₃	-H	8	55	190-194	C ₂₇ H ₁₉ N ₃ O ₂ S	0.62
6	-H	-CH ₃	10	40	165-167	C ₂₇ H ₁₉ N ₃ O ₂ S	0.56
7	-H	-H	8	55	142-145	C ₂₀ H ₁₃ N ₄ O ₂ S	0.66
8	-CH ₃	-H	10	53	150-152	C ₂₂ H ₁₅ N ₄ O ₂ S	0.59
9	-H	-CH ₃	12	49	132-135	C ₂₂ H ₁₆ N ₄ O ₂ S	0.54
10	-H	-H	10	58	153-155	C ₂₆ H ₂₂ N ₂ O ₅ S	0.59
11	-CH ₃	-H	12	50	166-168	C ₂₈ H ₂₄ N ₂ O ₅ S	0.60
12	-H	-CH ₃	15	45	175-178	C ₂₈ H ₂₄ N ₂ O ₅ S	0.57

R¹, R² = Substitution of groups.
R_f = Retention factor.

Table 1: Physicochemical data of synthesized compounds [4-12].

Compounds	Diameter of zone of inhibition (mm) Mean ^a		
	<i>C. albicans</i>	<i>A. niger</i>	<i>P. citrinum</i>
4	9.57	6.42	5.64
5	10.42	8.90	--
6	11.06	--	6.76
7	16.78	12.55	13.81
8	12.43	10.12	8.90
9	11.82	8.65	9.78
10	10.72	10.29	9.52
11	11.48	8.87	8.38
12	9.78	9.05	--
Fluconazole	23.18	22.32	20.71
DMSO	00	00	00

^a Mean value of measured diameters of zones of inhibition at 200 µg/ml for test compounds.

Table 2: Antifungal activity of synthesized compounds.

5-(3,4,5-trimethoxybenzylidene)-2-thioxo-1,3-di-p-tolyl-dihydropyrimidine-4,6(1H,5H)-dione (Scheme 1(12)).

Pale yellow; IR (KBr) cm⁻¹: 3245 (NH str.), 2923, 2839 (C-H), 1669 (C=O), 1541 (C=C), 1247 (C-N), 1049 (C=S). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 3.53 (s, 9H, 3×OCH₃), 7.11-7.15 (m, 2H, H_{methoxyphenyl}), 7.37-7.56 (m, 8H, H_{phenyl}), 8.39 (s, 1H, =CH-) (Scheme 1).

Antifungal activity

The newly synthesized substituted aldehyde containing derivatives of thiobarbituric acids were screened for their antifungal activity against three different moulds by cup-plate method [22]. Potato dextrose agar (PDA) was used as culture medium. Normal saline with tween 80 (0.01%) used to make suspension of fungal spore for lawning. Fifty milliliters of PDA medium at temperature between 40°C to 45°C was poured into each petri dish (15 cm diameter) and allowed to set. 5 ml of the spore suspension was spread over the solid agar medium and plates were kept in incubator at 37°C for 1 h. With the help of an agar punch, wells were made on these seeded agar plates and dilutions of test compounds in Dimethyl sulfoxide (DMSO) were added into each well, labeled previously. A control was also prepared using solvent DMSO. The petri plate were prepared in duplicate and maintained at 30°C for 72 hrs. Antifungal activity was determined by measuring zone of inhibition in millimeter and results were reported as (Mean zone of inhibition) [23,24]. Activity of each compound was compared with standard Fluconazole and results have been summarized in Tables 1 and 2.

Results and Discussion

Chemistry

The synthesis of compounds 4-12 were undertaken as per the

sequence of reaction outlined in Scheme 1. The required thiobarbituric acid derivatives were prepared by the Knoevenagel condensation of 1,3-diphenylthiobarbituric acid with substituted carboxaldehyde derivatives in absolute alcohol using piperidine as a base. The synthetic route for the final compounds starts with the formation of substituted biphenyl thiourea from reaction of carbon disulfide and substituted aniline in ethanol. Then the cyclization of substituted biphenyl thiourea in the presence of malonic acid and acetyl chloride afforded substituted biphenyl thiobarbituric acid. The subsequent condensation of substituted TBA with different carboxaldehyde derivatives yielded final compounds summarized in Table 1. All the final compounds were characterized by IR, ¹H NMR spectroscopy. They were screened for their antifungal activity against *C. albicans*, *A. niger* and *P. citrinum*. Fluconazole was used as standard.

Antifungal activity

Titled compounds (Scheme 1(4-12)) were evaluated for their antifungal potential and the results have been summarized in Table 2. Among the test compounds in the present series pyrazole containing derivatives of Thiobarbituric acid (Scheme 1(7-9)) were found active with increased zone of inhibition (mm) against all the tested moulds. Compound 7 (Figure 1) emerged as potent antifungal agent and its measured zone of inhibition (mm) against the tested strains, *C. albicans* (16.78), *A. niger* (12.55) and *P. citrinum* (13.81), were almost more than 50% than those obtained from the standard drug Fluconazole. It has also been observed that the unsubstituted biphenyl thiobarbituric acid derivatives were found more active than the substituted biphenyl thiobarbituric acid derivatives.

Conclusion

The antifungal activity data showed that all the newly synthesized compounds have moderate to good activity at 200 µg/ml concentration against *C. albicans*, *A. niger* and *P. citrinum*. It has been concluded that in the present series pyrazole derivatives of thiobarbituric acid (Scheme 1(7-9)) were found more active than the indole and 3, 4, 5-trimethoxyphenyl derivatives against all the tested moulds. 5-[(5-chloro-3-methyl-1H-pyrazol-4-yl) methylidene]-1, 3-diphenyl-2-thioxodihydro pyrimidine-4, 6(1H, 5H)-dione (Scheme 1(7)) emerged as potent antifungal agent among all the test compounds. Antifungal activity of active compounds may be attributed due to the presence of sulfur instead of carbonyl oxygen at 2nd position that incurs higher lipid solubility. Moreover, the unsubstituted biphenyl thiobarbituric acid derivatives were found more active than the substituted biphenyl thiobarbituric acid derivatives.

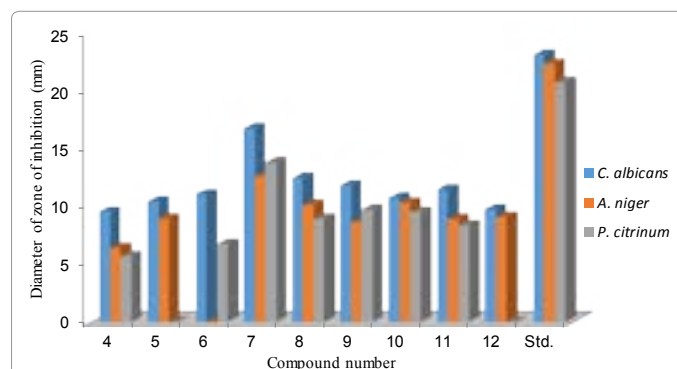


Figure 1: Antifungal activity of newly synthesized compounds [Refer Scheme 1 (4-12)].

References

1. Brouwer, W.G., Felauerand, E.E., & Bell, A.R. Study of antioxidant properties of the newly synthesized hydroxybenzylidene barbituric/thiobarbituric acid (HBBA/HBTBA) derivatives and their reduced forms all containing azo moiety. U.S. Patent 1990, **982**:779. Chem. Abstr. 1991, **114**:185539.
2. (a) Dabholkar, V.V & Tripathi, D.R. Synthesis of *Biginelli* products of Thiobarbituric acids and their antimicrobial activity. J. Serb. Chem. Soc. (2010), **75 (8)** :1033–1040
(b) Akopyan, L.K., Adzhibekyan, A.S., Porkinyan, G.A. & Tumasyan, E.A. Bilzh, Arm. 1976, **29**:80, Chem. Abstr. 1976, **85**: 72068.
3. (a) Pareek, D., Chaudhary, M., Pareek, P.K., Kant, R., Ojha, K.G. & Pareek, A. Synthesis of some biologically important 2- Thiobarbituric acid derivatives incorporating benzothiazole moiety. Der Pharmacia Lettre, 2010, **2(4)**: 274-283.
(b) Katz, S.L., & Gay, A.W. U.S. Patent Chem. Abstr., 1982, **352**: 806. 1983, **98**: 215603.
4. Rajitha, G., Ravibabu, V., Ramesh, G. & Rajitha, B. Synthesis and antimicrobial activity of novel imidazo[1,2-a]pyridinopyrimidine-2,4,6(1H,3H,5H)-triones and thioxopyrimidine-4,6(1H,5H) diones. Res Chem Intermed. 2016, **42(3)**: 1989–1998.
5. Thakur, R., Mohan, R. & Kidwai, M. Ecofriendly synthesis of novel antifungal (Thio) barbituric acid derivatives. Acta chim.solv. 2005, **52**: 88-92.
6. (a) Taylor, J.B. Tandem Aldol-Michael reaction in aqueous diethylamine medium: a greener and efficient approach to bis- pyrimidine derivatives. Int J Mol Sci. 2013, **14(12)**: 23762–23773.
(b) Williams, D.A., & Lemke, T.L. Foye's Principles of Medicinal Chemistry, (5th edn) Lippincott Williams and Wilkins, New York, 2002.
7. (a) Ashkinazi, R.I. Microwave assisted synthesis of barbituric and thiobarbituric acid derivatives. PCT Int Appl WO 99/25,699; Chem Abstr 1999, **131**: 5267.
(b) Morgan, L.R., Juraic, B.S., Hooper, C.L., Naumann, D.M., Thangaraj, K. & Blanc, B.L., Bioorg Med Chem Lett, 2002, **12**: 3407.
8. Brown, D.J., Katritzky, A.R. & Rees, C.W. Synthesis of heterocyclic compounds using carbon disulfide and their products. Comprehensive Heterocyclic Chemistry, 1984, **3** :57.
9. Sachar, A., Gupta, P., & Sharma, R.L. Synthesis of some novel barbituric acid and 1,3- cyclohexanedione based condensed heterocycles. Indian Journal of Chemistry 2009, **48B**:1187-1194.
10. Andeani, A., Rambaldi, M., Locatelli, A., Leoni, A., Bossa, R., Chiercozzi, M., Galatulas, I. & Salvatore P. Synthesis of lactams with potential cardiotoxic activity. Eur J Med Chem, 1993, **28** :825.
11. Rabbani, M., Wright, E.J., & Little, H.J. Tolerance to competitive NMDA antagonists, but no cross tolerance with barbiturates. Pharmacol Biochem Behav, 1995, **50**: 9.
12. (a) Bikker, J.A., Kubanek, J., & Weaver, D.F. Quantum pharmacologic studies applicable to the design of anticonvulsants: Theoretical conformational analysis and structure-activity studies of barbiturates. Epilepsia, 1994, **35**:411.
(b) Andrews, P. R., Mark, L. C., Winkler, D. A., & Jones, G. P. Structure-activity relationships of convulsant and anticonvulsant barbiturates: A computer- graphic-based pattern-recognition analysis. J Med Chem. 1983, **26**:1223.
13. Windholz M. The Merck Index, Windholz M, (Ed) 10th edn. Merck and Co., Rahway, New Jersey, 1983.
14. Habibi, A. & Tarameshloo, Z. A new and convenient method for synthesis of Barbituric acid derivatives. J. Iran .Chem. Soc., 2011, **8(1)**: 287-291.
15. Gholamhassan, I., Kabiri, S., & Taghavi, S. Solvent free C-alkylation of barbituric acid in the nanocrystalline mordenite media. J.Chil. Chem. Soc. 2013, **58 (3)**: 1888-1891.
16. Wolff, M.E. Burger's medicinal chemistry and drug discovery. Wiley, New York, 1997.
17. Oliva, A., & Zimmermann, G. Barbituric acid derivatives with antimetastatic and antitumor activity. International Patent WO **98**: 58925.
18. Kauffman, G.B. Anti-cancer uses for barbituric acid analogs. Journal of Chemical Education. 1980, **57**: 222.
19. Bojarski, J.T., Mokrosz, J.L., Barton, H.J. & Paluchowska, M.H. The quantitative analysis of steric effects in heteroaromatics. Adv Heterocycle Chem, 1985, **38**: 229.
20. Ahluwalia, V.K., Bhagat, P., Aggarwal, R., & Chandra, R. Intermediates for organic synthesis. I.K. International Pvt. Ltd., 2005, **64**: 282-284.
21. Kumar, R.N., Suresh. T., & Mohan, P.S. Synthesis and antibacterial activities of newer derivatives of pyrido [2,3-d; 6,5-d'] dipyrimidines. Indian Journal of Chemistry, 2003, **42B**: 1734-1737.
22. Siddiqui, N., & Shamsuzzaman. Single-step synthesis, Characterisation and Antimicrobial studies of novel steroidal 1', 2', 3'- Thiadiazoles. J.Chil. Chem. Soc. 2013, **58 (3)** : 1934-1936.
23. Barry, A.L. The antimicrobial susceptibility test: Principle and practice. Biol. Abstr. 1977, **64**: 25183.
24. Suresh, M., Lavanya, P., Sreekanth, B., Jonnalagadda & Rao, C.V. Synthesis and biological studies of novel 2-(4- substituted benzyl-thio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido-[2,3-d]-pyrimidin-4(3H)-one derivatives. Org. Commun., 2011, **4(2)**: 33-41.