Research Article

An Ecofriendly Synthesis, Molecular Docking and Antimicrobial Evaluation of 5-Arylidyne 2-Thiobarbituric Acid Derivatives

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ABSTRACT

A series of 5-arylidine thiobarbiturates were synthesized in aqueous medium using tamarind juice as used as a natural catalyst, in yields of 75–85%. The target compounds were characterized by IR, NMR and MASS. There *in vitro* antifungal activity was evaluated by agar well diffusion method. All the target compounds exhibited good activities against fungal species. Molecular modeling studies were performed to dock compounds into the Lectins binding site, which suggested probable inhibition mechanism. The difference in activities between the synthesized moieties influenced by the substituents which provided several hints for the further investigation on structural modifications.

Keywords: 5-arylidine thiobarbiturates, molecular docking, antifungal activity.

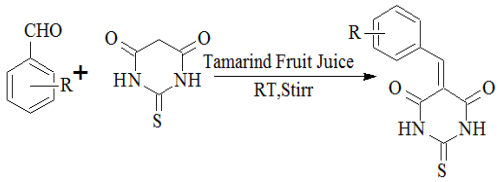
INTRODUCTION

In the past two decades, the classic organic chemistry has been rewritten around new approaches that investigate for the perfection of environmentally safer products[1-2]. Improvement of safe synthetic methodologies for organic reactions is one of the most recent challenges to the organic chemists[3]. The growing concern for the environment demands, the development of eco-friendly and economic process of safe organic synthesis[4-6]. Tamarind fruit juice as natural green catalyst, it is easily available and due to its acidic nature (pH=3) has been found to be a suitable interchange for various homogeneous acid catalysts, which can be used as biocatalyst in the organic transformations and synthesis in a too easy and environmentally friendly manner.

Development of novel synthetic methodologies to facilitate the preparation of desired molecule is an intense area of research[7-10]. In this regard, efforts have been made constantly to introduce new methodologies that are efficient and more compatible with the environment. Recently various strain resistant drugs failed to develop their results due to the unavailability of the knowledge gained from the morphological variations of micro-organisms[11-12].

The thiobarbituric acid scaffold consists of a pyrimidine cyclic structure. These compounds have been described as privileged structures, as they provide various points of attachment for a diverse array of structural elements that can be used to target receptor agonists or antagonists owing to the versatile these compounds are more often used for the man kind ailment. Most of the thiobarbiturate derivatives possessed a wide range of biological application in pharmaceutical as well as agrochemicals such as anti-inflammatory, antioxidant, antidepressant, antitumor, antibacterial, sedative, herbicides, fungicidal and antiviral agents[13-15] etc. Molecular modeling is one of important tool that shows exact active site of molecule in pharmacophore[16-17], therefore, there is a great demand for eco-friendly product which is easily degradable into the nontoxic residue harmless to human being and moreover beneficial to the crop. Led by these considerations the need for novel antimicrobial agents that exhibit broad spectrum and good water solubility has become more pressing.

In the light of the aforementioned facts and the demand for increasingly clean and efficient drug moieties, our interest in the synthesis of biologically active heterocyclic compounds, herein we report the synthesis, molecular docking and biological evaluation of 5-arylidyne 2-thiobabrbituric acid derivatives using tamarind fruit juice as natural catalyst (Scheme1).



MATERIALS AND METHODS

The reagent grade chemicals were obtained from commercial sources and used without further purification. Purity of synthesized compounds has been checked by thin layer chromatography. Melting points were determined by open capillary method and are uncorrected. Infrared spectra were recorded as on a Perkin-Elmer FTIR spectrometer and result are report in cm^{-1}. ^{1}H and ^{13}C NMR spectra was recorded on Bruker Avon 300MHz spectrometer using DMSO-d6 as solvent

and TMS as internal standard. Chemical shifts are expressed as δ values (ppm).

Experimental Procedure

General Procedure for Extraction of Tamarinds' indica Fruit Juice

The upper shell and inner grain of unripe tamarind fruit were removed with the help of a knife. The hard green material (pulp, 10 g) was boiled with water (50 mL), cooled and it was centrifuged. The clear portion of the aqueous extract (pH=3) of tamarind fruit was used as catalyst for the reaction.

General Procedure for the Condensation of 2-Thiobarbituric acid with aryl aldehydes

In a clean round bottomed flask, a mixture of 2-thiobarbituric acid (1mmol) and aryl aldehyde (1mmol) in tamarind fruit juice (1-2ml) was stirred at room temperature till the completion of reaction as monitored by TLC. After the completion of reaction the resulting crude product was filtered off, washed with water. The solid was dried and recrystallized from ethanol to obtain desired product an excellent yield.

Spectral data of Selected compounds

5-[4-(methylsulfanyl) benzylidene]-2-thioxodihydropyrimidine-4, 6(1H, 5H)-dione (5b)

Orange powder, yield 80%, mp 284°C; IR (KBr, cm^{-1}): 3070, 2906, 1689, 1650. ^{1}H NMR (300 MHz, DMSO-d6): δ 2.35(s, 3H), 5.809(s, 1H), 6.95-6.98(d, 2H.J=9Hz), 7.00-7.034(d, 2H.J=10Hz), 12.23(s, 1H), 12.33(s, 1H) ppm.

5-benzylidene-2-thioxodihydropyrimidine-4, 6(1H, 5H)-dione (5a)

Yellow powder, yield 75%, mp 263°C; IR (KBr, cm^{-1}):3416, 3085, 1685, 1591. ^{1}H NMR (300 MHz, DMSO-d6): δ 5.89(s, 1H), 7.02-7.05(d, 3H J=9Hz), 7.11-7.16(dd, 2H), 11.88(d, 2H)ppm.

5-(pyridin-4-ylmethylidene)-2-thioxodihydropyrimidine-4, 6(1H, 5H)-dione(5c)

Faint orange powder, yield 75%, mp 270°C; IR (KBr, cm^{-1}):3158, 2842, 1684, 1628. ^{1}H NMR (300 MHz, DMSO-d6): δ 6.19(s, 1H), 7.69(d, 2H), 8.66(d, 2H), 11.80(s, 1H)ppm.

Table 1: Physical Data of Synthesized Compounds (1a-1j)

Compound	R	Mol Formula	Colour	Yield
5a	C_6H_5	$C_{11}H_8N_2O_2S$	Faint Yellow	80%
5b	P-SCH3	$C_{12}H_{10}N_2O_2S_2$	Dark Orange	78%
5c	Pyridyl	$C_{10}H_7N_3O_2S$	Yellow	75%
5d	P-Cl	$C_{11}H_7ClN_2O_2S$	Whitish Yellow	82%
5e	O-Cl	$C_{11}H_7ClN_2O_2S$	Faint Yellow	81%
5f	P-CH3	$C_{12}H_{10}N_2O_2S$	Yellow	76%
5g	P-OCH3	$C_{12}H_{10}N_2O_3S$	Yellow	75%
5h	M-Br	$C_{11}H_7BrN_2O_2S$	Faint Orange	70%
5i	Thiopene	$C_9H_6N_2O_2S_2$	Greenish Yellow	78%
5j	P-OH	$C_{11}H_8N_2O_3S$	Yellow	80%

The Table 1 above represents the list of synthesized 5-arylidine thiobarbituric acid derivatives and their physical characterisation.

***In-vitro* Antifungal Activity Study**

All the solution of the target compounds, were prepared concentrations as 100, 200 and 300ppm in DMSO were tested in vitro against three pathogenic fungi and using agar well diffusion method and the zone of inhibition (Mean ± SD) are shown in comparison with that of standard Benomyl and bavistin in Table 1. The *in vitro* antifungal activity was carried out against *Sclerotium rolfsii, Trichosporon beigelii, and Tricthothecium sp.* The inhibition zone that appeared after 48 h, around the well in each plate were measured as zone of inhibition in mm. Experiments were performed in triplicates and standard deviation were calculated.

***In silico* Molecular Docking**

Ligand Preparation

The structure of 2-Thiobarbituric acid was used as the template to build the molecules in the dataset in builder module of Vlife MDS 4.3. The geometry and energy of the molecules were further optimized by the optimization module of the V life MDS 4.3 using Merck molecular force field.

Docking Studies

Docking simulations was carried out using Biopredicta module of Vlife MDS 4.3 using crystal structure of Sclerotium rolfsii Lectin (PDB ID 2OFD) downloaded from www.rcsb.org.

Protein Preparation

The protein preparation is an important step in the virtual screening. The selected Sclerotium rolfsii Lectin was prepared using biopredicta module of the V Life MDS 4.3. The water molecules were removed and hydrogens were added in to the lectin structure to retain the geometry, and this structure was optimized by the optimization module of the V life MDS 4.3 using Merck molecular force field.

Pharmacophore Identification Studies

Pharmacophore modeling was also carried out in V life MDS 4.3 using Mol sig module. The minimum number of pharmacophore features generated for an alignment is taken 4 and tolerance is kept to 10 Å. The max distance allowed between two features is kept to 10 Å.

RESULTS AND DISCUSSION

The synthesis of 5-arylidine thiobarbituric acid derivatives was achieved conventional method. However, these moieties were prepared using natural acid as tamarind fruit juice with short period of time at room temperature to obtain good yields in75-85% with high purity. The investigation of antifungal screening revealed that some of the tested compounds showed moderate to good

antifungal activity against all pathogens shows as using *in silico* molecular docking study.

To evaluate the overall antimicrobial activity of 5-arylidine 2-thiobarbiturates, representative pathogen was selected as the targets.

The testing results against three fungal pathogen were shown that the all synthesized moieties are active (listed in Table 1). For comparative purposes, the title compounds 1b, 1f and 1e shows excellent antifungal activity against *Sclerotium rolfsii, Trichosporon beigelii, and Tricthothecium sp as* compared to Benomyl and Bavistin.

However the compound **1a, 1d and 1h showed as moderate activity.** The activity results were compared with benomyl, bavistin and summarized in Table 2.

Comp	*Sclerotium rolfsii (mm)*			*Trichosporon beigelii (mm)*			*Tricthothecium (mm)*		
	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
1a	20	23	23	18	19	20	19	18	20
1b	22	23	24	17	20	21	21	22	24
1c	21	22	23	18	20	21	20	21	22
1d	20	23	23	16	19	20	19	21	23
1e	19	21	20	18	17	20	20	22	21
1f	19	21	22	19	20	22	19	21	23
1g	21	23	24	18	20	21	19	20	23
1h	19	20	21	17	19	21	18	19	22
1i	18	19	22	18	21	22	20	21	23
1j	18	19	20	16	18	19	17	19	20
Bavistin	26	31	32	25	27	28	29	32	34
Benomyl	27	30	31	24	26	29	28	30	32

Molecular Docking Studies

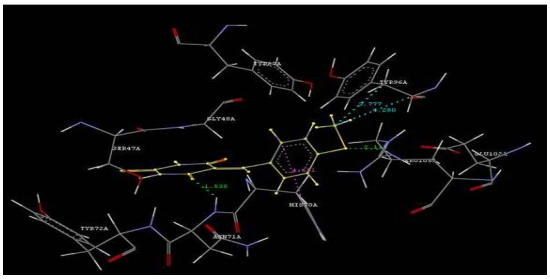


Figure 2: Figure showing best fitted designed inhibitor in *Sclerotium rolfsii* lectin.

The docking analysis helps to identify the mechanism of action of targeted derivatives and also gives an idea about the further investigations.

To identify the possible mechanism of action for the antifungal activity the docking analysis was carried out using the crystal structure of the *Sclerotium rolfsii* lectin.

Lectins are the group of protein originated from non immune origin playing vital role in the pathogenesis and other important biochemical role of the fungi. All the compounds showed nice fit in the active site of Sclerotium rolfsii lectin.

The derivatives are founds to showing hydrogen bond interactions with ARG105(2.1A0), ASN71(1.5 A0) and aromatic interaction with HIS70(4.6A0) and hydrophobic interactions TYR96(3.7A0)while a number of vander wall interactions with TYR27, SER47, GLY48, HIS70, ASN71, TYR72, GLU102, ARG105 as shown in Figure no 2.

Pharmacophore Modeling

Pharmacophore is important concepts in computational chemistry which is nothing but the group of features which are responsible for the activity these features normally include hydrogen bond donor, hydrogen bond accepter, positive & negative ionizable, hydrophobic, aromatic and aliphatic.

The pharmacophore modeling of the 5-arylidene 2-thiobarbiturates resulted in to the four point pharmacophoric hypothesis.

The features which are responsible for the antifungal activity of the 5-arylidene 2-thiobarbiturates are two hydrogen bond donor (green), hydrogen bond acceptor (violet) and aromatic feature (golden brown) as show in Figure 3 and 4.

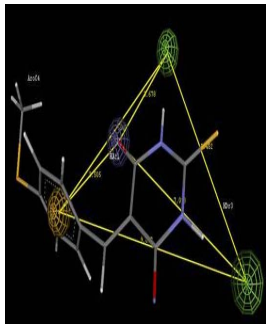


Figure 3: Figure showing selected pharmacophoric hypothesis

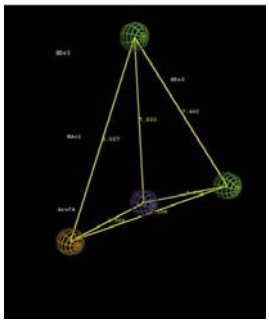


Figure 4: Figure showing pharmacophoric feature of designed inhibitors

CONCLUSION

A simple, quick and green and proficient method for the synthesis of 5-arylidene 2-thiobarbituric acid derivatives by condensation of substituted aryl aldehydes and 2-thiobarbituric acid in presence of tamarind fruit juice has reported. Ease of separation of pure product, selectively and in an excellent yield.

Among the screened samples, compound **1b, 1f and 1e** has emerged as most active against all tested microorganisms compared to the standard drug.

Finally, the molecular docking studies of the synthesized compounds were carried out and the results of such studies were reported. *In silico* studies revealed that all the synthesized compounds R-01 to R-08 have relatively lesser binding energy as compared to the standard drug and may be considered as a good inhibitor Lectins binding

site. Thus this study has widened the scope for developing these derivatives as the promising antifungal agents.

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REFERENCES

1. Patil S., Jadhav S and Deshmukh. M, Natural Acid Catalyzed Multi-component Reactions as a Green Archives of Applied Science Research, 3(1), 2011, 203-208.
2. Patil S., Jadhav S and Patil U., Natural Acid Catalyzed Synthesis of Schiff Base under Solvent-free Condition: As a Green Approach Archives of Applied Science Research, 4(2), 2012, 1074-1078.
3. Faida H., Bamanie A., Shehata A. S., Moustafa M. A., Mashaly M. M., Green chemistry 1: Simple and Efficient Synthesis-in Water-and Antibacterial Activity of 5-Arylidene Derivatives of Thiobarbituric and Barbituric acids, Journal of American Science, 8(1), 2012.
4. Bararjanian M., Balalaie S., Movassagh B. and Amani B., One-pot synthesis of pyrano[2,3-d]pyrimidinone derivatives catalyzed by L-proline in aqueous media, J. Iran. Chem. Soc, 6(2), 2009, 436-442.
5. Menegatti R., Ramesh E., Raghunathan R., Microwave-Assisted K-10 Montmorillonite Clay–Mediated Knoevenagel Hetero-Diels–Alder Reactions: A Novel Protocol for the Synthesis of Polycyclic Pyrano[2,3,4-kl]xanthene Derivatives Syn. Comm, 39, 2009, 613.
6. Habibi D., Marvi O., Montmorillonite KSF and montmorillonite K-10 clays as efficient catalysts for the solvent less synthesis of bismaleimides and bisphthalimides using microwave irradiation, Arkivok, xiii, 2006, 8.
7. Harjani J. R., Nara S. J., Salunkhe M. M., Lewis acidic ionic liquids for the synthesis of electrophilic alkenes via the Knoevenagel condensation, Tetrahedron Lett, 43, 2002, 1127–1130.
8. Khan F. A., Dash J., Satapathy R., Upadhyaya S. K., Hydrotalcite Catalysis in Ionic Liquid Medium: A Recyclable Reaction System for Heterogeneous Knoevenagel and Nitroaldol Condensation, Tetrahedron Lett, 45, 2004, 3055–3058.
9. Prajapati D. and Gohain M., An efficient synthesis of novel pyrano [2,3-d]-and furopyrano [2,3-d] pyrimidines via indium-catalyzed multi-component domino reaction, Beilstein Journal of Organic Chemistry, 2, 2006, 11.
10. Narsaiaha A. V. and Nagaiah K., An Efficient Knoevenagel Condensation Catalyzed by $LaCl_3.7H_2O$ in Heterogeneous Medium Synth. Commun, 33, 2003, 3825.
11. Ren Y. M. and Cai C., Knoevenagel Condensation of Aromatic Aldehydes with Active Methylene Compounds using a Catalytic Amount of Iodine and K_2CO_3 at Room Temperature, Synth. Commun, 37, 2007, 2209.
12. Su C., Chen Z. C., Zheng Q. G., Organic Reactions in Ionic Liquids: Knoevenagel Condensation Catalyzed by Ethylene diammonium Diacetate, Synthesis 2003, 555.
13. McCluskey A. J., Philip, Robinson, Tim Hill, Janet L., Scott C and Kate Edwards A, Green chemistry approaches to the Knoevenagel condensation: comparison of ethanol, water and solvent free (dry grind) approaches, Tetrahedron Letters. 43, 2002, 3117–3120.
14. Kadoma Y. and Fujisawa S., Radical-Scavenging Activity of Thiols, Thiobarbituric Acid Derivatives and Phenolic Antioxidants Determined using the Induction Period Method for Radical Polymerization of Methyl Methacrylate, Polymers. 4, 2012, 1025-1036.
15. Sahu D., Ghosh G., Sahoo J. Sudhir Kumar, Evaluation of Antimicrobial Activity of some newly Synthesized AZO Compounds Derived from Thiobarbituric Acid, International Journal of Advanced Chemical Science and Applications, 1, 2013, 25-27.
16. Gaber H. M., Abdel-Hafiz I. S., El-Sawy. K. M., and Sherif. S. M., New Fused Pyrimidines of Potential Biosignificant Interest. Syntheses and Molecular Modelling Studies, Acta Chim. Solv, 57, 2010, 230.
17. Zhi H., Chen L., Zhang L., Liu S., David Chi Cheong Wan, Huang-quan Lin, and Chun Hu, Design, synthesis, and biological evaluation of 5H-thiazolo[3,2-a]pyrimidine derivatives as a new type of acetylcholinesterase inhibitors, ARKIVOC xiii, 2008, 266-277.

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Prof. Deshmukh did B.Sc, M.Sc & Ph.D. from Shivaji University & guided 31 Ph.D, 11 M.Phil's and published 264 papers in reputed good impact factored journals. He was Post- Doctoral fellow 1993-94 in University of Graz, Austria & visiting Professor, Hanyang University, Korea during March2010. He was handled multiple UGC, DST projects at University.