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RESEARCH ARTICLE

SYNTHESIS AND *IN VITRO* STUDY OF NEW THIAZOLIDINONES WITH BARBITURIC ACID SCAFFOLD AS ANTIMICROBIAL AGENTS

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ABSTRACT

A series of new thiazolidinones with barbituric acid scaffolds 5(a-j) were synthesized and evaluated for their antibacterial activity against Gram-positive bacteria (*B. subtilis*, *B. sphaericus*, *S. aureus*), Gram-negative bacteria (*P. aeruginosa*, *K. aerogenes*, *C. violaceum*) and also antifungal activity against *C. albicans*, *A. fumigatus*, *T. rubrum* and *T. metagrophytes*. The antibacterial activity of compounds containing 4-hydroxyphenyl (5c), 2,6-difluorophenyl (5g) and 4-hydroxy-3-methoxy-phenyl (5i) groups on thizolidionone ring showed considerable activity against tested bacterial strains. Compound, contain 4-chlorophenyl group (5c) and 4-*N*,*N*-dimethylaminophenyl (5h) showed better activity against Gram-positive (except *B. Subtilis)* and Gram-negative bacteria (except *C. Violaceum)*. Similarly, compounds containing 4-methylphenyl (5b), 3-nitrophenyl (5f) and pipernyl (5j) were active against *B. sphaericus, and S. Aureus, respectively*. The antifungal evaluation of 5(a-j) indicates that the compounds 5e and 5i which contain 4-hydroxyphenyl and 4-hydroxy-3-methoxyphenyl respectively showed considerable activity against all the fungal strains.

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INTRODUCTION

The nitrogen and sulphur containing heterocyclic compounds are biologically significant, because many biologically potent compounds contain these heterocyclic rings such as thiamine, penicillin, and other antibiotics (James, 1966, Ghazzi, 1977) contain the thiazole ring. Similarly, thiazolidinone and its derivatives were also important and shows desired biological and pharmacological activities including hypnotic (Ergenc, 1999), antiinflamma- tory (Taranalli, 2008), antibacterial (Aakash, 2016), antifungal (Marques, 2014), antitubercu- lar (Sambhaji, 2017), anticancer (Dmytro, 2010), antitumor (Dmytro, 2012), analgesic (Vishnu, 2009), anesthetic (Vicini, 1990), antiproliferative (de Oliveira, 2017), anti-HIV (Ravichandran, 2009) and nematicidal (Srinivas, 2008). Furthermore, thiazolidinones have been used for the treatment of schizophrenia (Hrib, 1992), diabetic complications like cataract, nephropathy, neuropathy (Lesyk, 2004) and selective antiplatelet activating factor (Tanabe, 1995). Moreover, the thiazolidinone derivatives are also employed in the synthesis of cyanine dyes, which are used in photographic film industry (Abd E1-Aal, 2003).

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Similarly, barbituric acids play an important role in many drugs with biological and pharmaceutical properties (Trost, 2000). Although barbituric acid itself is hypnotically inactive, its derivatives substituted at C-5 are reported as central nervous system depressants (Goel, 2005), sedative, hypnotic (Joseph, 1986), anesthetic (Md. Ehsanul, 2015), anticonvul-sants (Shabnam, 2018), anxiolytic agents (Farshid, 2012). Clinically important hypnotic-sedative barbiturates have substitutions at 5th position of barbituric acid (Francisco, 2005) and the also side chains at 5th position is essential for hypnotic activity (Meir, 2012). Inspired by the biological properties of thiazolidinone and barbituric acid derivatives, and in continuation of our research on biologically active heterocycles (Nagaraj, 2017, 2018) it was thought of interest to couple thiazolidinone and barbituric acid heterocycles to a single structure for enhancing biological activity. The present investigation deals with the use of barbituric acid in the synthesis of some interesting and new thiazolidinones with barbituric acid scaffolds to study their effect on bacteria and fungi.

MATARIALS AND METHODS

All reagents are commercial grade and were used as supplied. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck, and compounds visualized by exposure to UV light.

Chromatographic columns 70–230 mesh silica gel for separations were used. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard and coupling constants (*J*) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

1,3-dimethyl-5-nitrohexahydro-2,4,6-pyrimidinetrione (2): To the stirred and cooled fuming HNO_3 (9.7 mL), 1,3-dimethylbarbituric acid **1** (0.05 mol, 6.8 g) is added over a period of two hours, the temperature is kept below 40 °C during the addition and the mixture is stirred for 1 h. Water is added and the solution is cooled to 10 °C. The mixture is filtered, and the residue is washed with cold water and dried on a glass try at 60-80 °C, recrystalised from hot water to gave compound **2**. Yield 52%; IR (KBr) ν_{max}: 2968, 2765, 1741, 1621, 1587, 1372 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97 (s, 6H, CH_3), 5.92 (s, 1H, CH-NO_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.9, 82.1, 149.7, 162.3; MS: *m/z* 201 (M^+).

5-amino-1,3-dimethylhexahydro-2,4,6-pyrimidinetrione (3): A mixture of 1,3-dimethyl-5-nitrobarbituric acid **2** (0.05 mol, 10.5 g) and conc. HCl (63 mL) is heated on a boiling water bath. To the hot solution, a mixture of tin (26.25 g) in HCl (42 mL) is gradually added over a period of 30 min. Heating is continued until there is no yellow colour persisted. The resulting white solid was taken up in HCl (50 mL), filtered, concentrated to half the volume, left overnight in the refrigerator, the white powdery solid collected, washed with dil HCl and then water and dried to pure product **3**. Yield 49%; IR (KBr) ν_{max}: 3366, 2970, 2762, 1739, 1622 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.87 (s, 6H, CH_3), 3.23 (s, 2H, NH_2), 4.12 (s, 1H, CH-NH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 54.3, 152.3, 169.8; MS: *m/z* 171 (M^+).

General procedure for the synthesis of 1,3-dimethyl-5-[(*E*)-pyrimidinetrione (4): To solution of compound **3(a-j)** (0.01 mol, 1.71 g) in toluene (20 mL), aromatic aldehyde (0.01 mol) and glacial acetic acid (0.5 mL) was added and refluxed the mixture for 3 h using a Dean–Stark apparatus and the water formed was removed azeiotropically. The progress of the reaction was checked by TLC using toluene: ethyl acetate (4:1) as an eluent. After completion of the reaction, solvent was removed by distillation to give the solid, which was filtered, and recrystallized from ethyl alcohol to get the pure compound 4(a-j).

1,3-dimethyl-5-[(E)-1-phenylmethylidene]aminohexahydro-2,4,6-pyrimidinetrione (4a): Yield 61%; IR (KBr) ν_{max}: 3068, 2955, 2267, 1741, 1618 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.87 (s, 6H, CH_3), 4.92 (s, 1H, CH-N), 7,00-7.05 (m, 3H, ArH), 7.76 (s, 1H, CH=N), 7.62 (d, *J* = 7.1 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.4, 66.1, 126.7, 129.6, 132.4, 133.6, 152.3, 163.9, 178.1; MS: *m/z* 259 (M^+).

1,3-dimethyl-5-[(E)-1-(4 aminohexahydro-2,4,6-(4b): Yield 67%; IR (KBr) ν_{max}: 3072, 2951, 2234, 1722, 1621 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.11 (s, 3H, CH_3), 2.82 (s, 6H, CH_3), 4.91 (s, 1H, CH-N), 6.92 (d, *J* = 7.4 Hz, 2H, ArH), 7.62 (s, 1H, CH=N), 7. 82 (d, *J* = 7.4 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.3, 23.4, 66.1, 127.7, 129.5, 133.1, 139.9, 152/3, 163.9, 178.1; MS: *m/z* 273 (M^+).

Chlorophenyl)methylidene]amino-1,3-dime thylhexahydro-2,4,6-pyrimidine- trione (4c): Yield 71%; IR (KBr) ν_{max}: 3071, 2942, 2218, 1729, 1618, 685 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81 (s, 6 H, CH_3), 4.87 (s, 1H, CH-N), 6.99 (d, *J* = 7.6 Hz, 2H, ArH), 7.67 (s, 1H, CH=N), 7.92 (d, *J* = 7.6 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.4, 66.1, 127.9, 129.0, 134.8, 138.2, 152.3, 163.9, 178.1; MS: *m/z* 293 (M^+).

1,3-dimethyl-5-[(E)-1-(4-thylidene] aminohexahydro-2,4,6-(4d): Yield 53$; IR (KBr) ν_{max}: 3052, 2944, 2221, 1729, 1618, 1582, 1367 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.91 (s, 6H, CH_3), 4.86 (s, 1H, CH-N), 7.66 (s, 1H, CH=N), 7.92 (d, *J* = 8.3 Hz, 2H, ArH), 8.07 (d, *J* = 8.3 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.3, 66.9, 117.6, 134.7, 136.9, 155.5, 167.6, 169.7, 177.2; MS: *m/z* 304 (M^+).

5-[(E) thylidene]amino-1,3-dimethylhexahydro-2,4,6-one (4e): Yield: 64%; IR (KBr) ν_{max}: 3374, 3066, 2934, 2229, 1742, 1642 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.91 (s, 6H, CH_3), 4.89 (s, 1H, CH-N), 5.23 (s, 1H, OH), 6.82 (d, *J* = 8.1 Hz, 2H, ArH), 7.47 (d, *J* = 8.1 Hz, 2H, ArH), 7.72 (s, 1H, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.3, 66.9, 112.8, 132.7, 133.9, 151.5, 161.6, 162.7, 177.8; MS: *m/z* 275 (M^+).

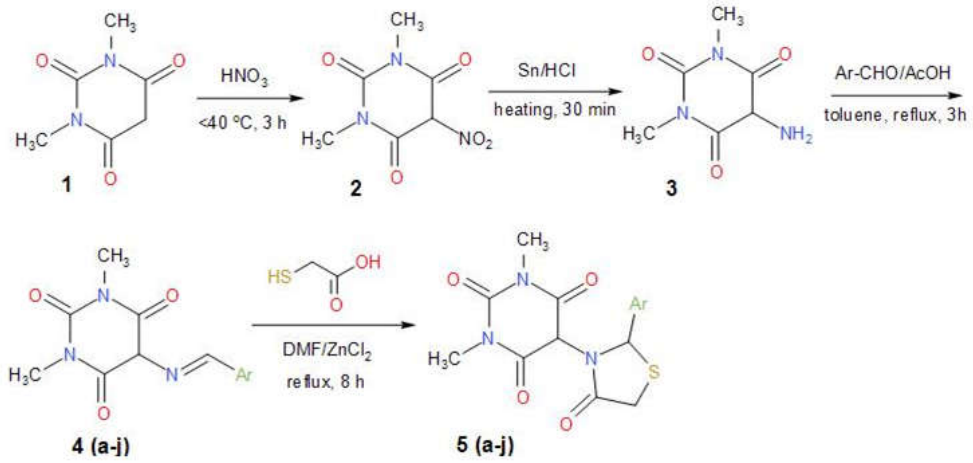
1,3-dimethyl-5-[(E)-1-(3-thylidene] aminohexahydro- 2,4,6-(4f): Yield 44%; IR (KBr) ν_{max}: 3051, 2941, 2228, 1723, 1623, 1571, 1362 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.90 (s, 6H, CH_3), 4.89 (s, 1H, CH-N), 7.65-7.80 (m, 3H, CH=N & ArH), 8.20-8.25 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.7, 66.2, 121.7, 124.0, 128.7, 131.1, 135.3, 146.9, 152.1, 172.5, 177.6; MS: *m/z* 304 (M^+).

5- [(E)-difluorophenyl) methylidene] amino-1,3-dimethylhexahydro-2,4,6-(4g): Yield 56%; IR (KBr) ν_{max}: 3037, 2980, 2232, 1725, 1617, 1132 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.85 (s, 6H, CH_3), 4.88 (s, 1H, CH-N), 6.77 (d, *J* = 8.2 Hz, 2H, ArH), 7.30-7.35 (m, 1H, ArH), 8.33 (s, 1H, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.0, 66.2, 106.8, 113.1, 132.6, 157.1, 163.7, 166.9, 177.7; MS: *m/z* 295 (M^+).

5-((E)-1-[4-(dimethylamino)phenyl]methylideneamino)-1,3-dimethylhexahydro-2,4,6-pyrimidinetrione (4h): Yield 69%; IR (KBr) ν_{max}: 3032, 2970, 2219, 1721, 1622 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.72 (s, 6H, CH_3), 2.92 (s, 6H, CH_3), 4.94 (s, 1H, CH-N), 6.18 (d, *J* = 8.6 Hz, 2H, ArH), 7.71 (s, 1H, CH=N), 7.92 (d, *J* = 8.6 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 40.2, 65.7, 110.5, 123.6, 127.9, 151.9, 153.4, 162.7, 177.7; MS: *m/z* 302 (M^+).

5-[(*E*)-1-(4-hydroxy-3-methoxyphenyl)methylidene]amino-1,3-dimethylhexahydro-2,4,6-pyrimidinetrione (4i): Yield 55%; IR (KBr) ν_{max}: 3387, 3037, 2912, 2237, 1724, 1629, 1072 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (s, 6H, CH_3), 3.72 (s, 3H, OCH_3), 4.72 (s, 1H, CH-N), 4. 86 (s, 1H, OH), 6.55 (d, *J* = 8.4 Hz, 1H, ArH), 7.12 (d, *J* = 8.4 Hz, 1H, ArH), 7.49 (s, 1H, ArH), 7.72 (s, 1H, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.1, 57.8, 67.2, 115.3, 118.8, 127.1, 128.9, 151.5, 152.4, 153.0, 161.7, 177.9; MS: *m/z* 305 (M^+).

5-[(*E*)-1-(1,3-benzodioxol-5-yl)methylidene]amino-1,3-dimethylhexahydro-2,4,6-netrione (4j): Yield 48%; IR (KBr) ν_{max}: 3087, 2989, 2232, 1729, 1618, 1122 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81 (s, 6H, CH_3), 4.90 (s, 1H, CH-N), 6.71 (d, *J* = 8.3 Hz, 1H, ArH), 6.91 (s, 2H, O-CH_2-O), 7.20-7.25 (m, 2H, ArH), 7.72 (s, 1H, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.1, 66.5, 99.6, 107.9, 110.9, 124.2, 126.1, 128.1, 148.9, 152.7, 153.4, 163.5, 178.3; MS: *m/z* 303 (M^+).



4/5: Ar = **a**) phenyl; **b**) 4-methylphenyl; **c**) 4-chlorophenyl; **d**) 4-nitrophenyl; **e**) 4-hydroxyphenyl; **f**) 3-nitrophenyl; **g**) 2,6-difluorophenyl; **h**) 4-*N*,*N*-dimethylaminophenyl; **i**) 4-hydroxy-3-methoxyphenyl; **j**) 3-pipernyl

Scheme 1. Schematic route for the synthesis of compounds 5(a-j)

Table 1. Antibacterial activity of compounds 5a-j

Compound	Minimum inhibitory concentration (MIC μg/mL)					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
5a	25.0	25.0	50.0	--	25.0	12.5
5b	25.0	12.5	--	50.0	25.0	--
5c	12.5	12.5	6.25	6.25	6.25	12.5
5d	25.0	--	12.5	50.0	25.0	--
5e	3.12	6.25	6.25	25.0	3.12	3.12
5f	12.5	25.0	--	50.0	3.12	12.5
5g	3.12	6.25	6.25	3.12	3.12	3.12
5h	25.0	12.5	6.25	3.12	3.12	25.0
5i	6.25	6.25	3.12	3.12	6.25	3.12
5j	25.0	12.5	6.25	25.0	--	25.0
Streptomycin	6.25	12.5	6.25	1.56	1.56	3.12

Note: – indicates, strains are resistant to the compound >50 μg/mL conc.

Table 2. Antifungal activity of compounds 5a-j

Compound	Minimum inhibitory concentration (MIC μg/mL)			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. metagrophytes*
5a	25.0	50.0	--	50.0
5b	12.5	25.0	12.5	--
5c	25.0	12.5	12.5	6.25
5d	25.0	12.5	25.0	--
5e	6.25	3.12	3.12	6.25
5f	12.5	6.25	25.0	12.5
5g	12.5	12.5	12.5	25.0
5h	12.5	25.0	25.0	--
5i	6.25	1.56	3.12	3.12
5j	12.5	12.5	25.0	12.5
Amphotericin B	6.25	3.12	3.12	3.12

Note: – indicates fungi are resistant to the compound >50 μg/mL conc.

General procedure for the synthesis of 1,3-dimethyl-5-(4-oxo-exahydro-2,4,6-pyrimidinetrione (5): A mixture of corresponding compound **4(a-j)** (0.01 mol), thioglycolic acid (0.012 mol) in *N*,*N*-dimethylformamide (20 mL) with a pinch of anhydrous $ZnCl_2$, was refluxed for 8 h.

The progress of the reaction was checked by TLC using toluene: ether (3:1) as an eluent. The reaction mixture was cooled to room temperature and then poured into crushed ice. It was set-aside for overnight at room temperature. The solid thus separated was filtered, washed several times with water, and purified by column chromatography on silica-gel with hexane-ethyl acetate as eluent to get the pure compound 5 (a-j).

1,3 -dimethyl-oxo-2 -phenyl-1, 3-thiazolan- 3-yl)-pyrimidinetrione (5a): Yield 39%; IR (KBr) ν_{max}: 3072, 2971, 2260, 1745, 1709, 1611, 1338, 878 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.87 (s, 6H, CH_3), 4.17 (s, 2H, CH_2-CO), 5.39 (s, 1H, CH-N), 5.69 (s, 1H, CH-S), 7.35-7.45 (m, 5H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.3, 35.4, 51.6, 67.2, 126.4, 128.7, 129.1, 139.6, 152.7, 167.9, 170.5; MS: *m/z* 333 (M^+).

1,3-dimethyl-5-[2-(4-methylphenyl)-4-oxo-1,3-thiazolan-3-yl]hexahydro-2,4,6-pyrimidine- trione (5b): Yield 41%; IR (KBr) ν_{max}: 3067, 2932, 2268, 1742, 1701, 1617, 1339, 872 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.22 (s, 4H, CH_3), 2.82 (s, 6H, CH_3), 4.14 (s, 2H, CH_2-CO), 5.37 (s, 1H, CH-N), 5.62 (s, 1H, CH-S), 7.10 (d, *J* = 8.3 Hz, 2H, ArH), 7.42 (d, *J* =

8.3 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.4, 24.7, 34.7, 52.3, 68.1, 124.4, 126.7, 133.9, 136.2, 151.6, 166.8, 169.4; MS: *m/z* 347 (M^+).

5-[oxo-1,3-thiazolan-3-yl]-1,3-dimethylhexahydro-2,4,6-one (5c): Yield 54%; IR (KBr) ν_{max}: 3063, 2931, 2260, 1739, 1710, 1611, 1323, 871, 689 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.88 (s, 6H, CH_3), 4.15 (s, 2H, CH_2-CO), 5.40 (s, 1H, CH-N), 5.66 (s, 1H, CH-S), 7.12 (d, *J* = 8.3 Hz, 2H, ArH), 7.42 (d, *J* = 8.3 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.3, 34.7, 52.3, 68.1, 127.7, 129.0, 133.7, 136.5, 153.1, 166.7, 169.6; MS: *m/z* 367 (M^+).

1,3- dimethyl-5-[2- (4-nitrophenyl)-4-, thiazolan-3-yl]hexahydro-2,4,6- (5d): Yield 61%; IR (KBr) ν_{max}: 3052, 2918, 2248, 1731, 1705, 1615, 1567, 1376, 1332, 871 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.84 (s, 6H, CH_3), 4.13 (s, 2H, CH_2-CO), 5.37 (s, 1H, CH-N), 5.67 (s, 1H, CH-S), 7.41 (d, *J* = 8.6 Hz, 2H, ArH), 7.61 (d, *J* = 8.3 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.2, 36.2, 52.7, 68.1, 122.7, 126.7, 145.9, 146.4, 153.1, 167.1, 171.2; MS: *m/z* 378 (M^+).

5-[2-(4-hydroxyphenyl)-4-oxo-1,3-thiazolan-3-yl]1,3-dimethylhexahydro-2,4,6-one (5e): Yield 45%; IR (KBr) ν_{max}: 3347, 3047, 2918, 2252, 1741, 1711, 1612, 1341, 870 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2. 85 (s, 6H, CH_3), 4.12 (s, 2H, CH_2-CO), 4.91 (s, 1H, OH), 5.33 (s, 1H, CH-N), 5.65 (s, 1H, CH-S), 6.72 (d, *J* = 8.1 Hz, 2H, ArH), 7.32 (d, *J* = 8.1 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.7, 34.3, 50.0, 68.1, 112.3, 124.7, 135.1, 152.0, 155.9, 166.8, 168.7; MS: *m/z* 349 (M^+).

1,3-dimethyl-5-[2-(3-nitrophenyl)-4-oxo-1,3-thiazolan-3-yl]hexahydro-2,4,6-one (5f): Yield 51%; IR (KBr) ν_{max}: 3046, 2918, 2256, 1735, 1714, 1615, 1578, 1377, 1333, 870 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.86 (s, 6H, CH_3), 4.20 (s, 2H, CH_2-CO), 5.37 (s, 1H, CH-N), 5.62 (s, 1H, CH-S), 7.40-7.45 (m, 2H, ArH), 8.10-8.15 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.1, 34.3, 53.7, 66.3, 118.3, 120.7, 128.3, 134.4, 139.7, 149.2, 151.6, 166.8, 171.1; MS: *m/z* 378 (M^+).

5-[2-(2,6-difluorophenyl)-4-oxo-1,3-thiazolan-3-yl]-1,3-dimethylhexahydro-2,4,6-etrione (5g): Yield 63%; IR (KBr) ν_{max}: 3046, 2919, 2234, 1732, 1709, 1612, 1341, 1132, 874 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (s, 6H, CH_3), 4.22 (s, 2H, CH_2-CO), 5.41 (s, 1H, CH-N), 5.71 (s, 1H, CH-S), 6.57 (m, 2H, ArH), 7.39 (m, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.2, 36.3, 53.1, 66.9, 114.7, 115.4, 129.9, 152.3, 162.7, 166.8, 170.7; MS: *m/z* 369 (M^+).

5-2-[4-(dimethylamino)phenyl]-4-oxo-1,3-thiazolan-3-yl-1,3-dimethylhexahydro-2,4,6-pyrimidinetrione (5h): Yield 54%; IR (KBr) ν_{max}: 3043, 2955, 2227, 1739, 1711, 1619, 1340, 871 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.86 (s, 6H, CH_3), 2.95 (s, 6H, CH_3), 4.15 (s, 2H, CH_2-CO), 5.32 (s, 1H, CH-N), 5.64 (s, 1H, CH-S), 6.27 (d, *J* = 8.5 Hz, 2H, ArH), 7.25 (d, *J* = 8.5 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.6, 35.7, 45.9, 52.1, 69.1, 114.7, 126.9, 136.7, 145.8, 152.1, 166.8, 169.8; MS: *m/z* 376 (M^+).

5-[2-(4-hydroxy-3-methoxyphenyl)-4-oxo-1,3-thiazolan-3-yl]-1,3-dimethylhexahydro-2, 4, 6-pyrimidinetrione (5i): Yield 65%; IR (KBr) ν_{max}: 3387, 3042, 2933, 2261, 1740, 1712, 1619, 1337, 1168, 871 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.85 (s, 6H, CH_3), 3.75 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2-CO), 4.82 (s, 1H, OH), 5.33 (s, 1H, CH-N), 5.64 (s, 1H, CH-S), 6.72 (d, *J* = 8.1 Hz, 1H, ArH), 7.25-7.30 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.7, 35.6, 52.1, 56.0, 68.1, 110.7, 119.4, 120.1, 136.9, 149.2, 150.7, 152.4, 166.8, 170.2; MS: *m/z* 379 (M^+).

5-[2-(1,3-benzodioxol-5-yl)-4-oxo-1,3-thiazolan-3-yl]-1,3-(5j): Yield 59%; IR (KBr) ν_{max}: 3032, 2952, 2263, 1737, 1713, 1622, 1328, 1118, 874 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.84 (s, 6H, CH_3), 4.23 (s, 2H, CH_2-CO), 5.41 (s, 1H, CH-N), 5.66 (s, 1H, CH-S), 5.80 (s, 2H, O-CH_2-O), 6.74 (d, *J* = 8.3 Hz, 1H, ArH), 7.30-7.35 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.9, 35.6, 51.4, 68.1, 102.7, 104.7, 110.7, 120.6, 136.7, 150.9, 151.1, 153.0, 166.7, 170.7; MS: *m/z* 377 (M^+).

RESULTS AND DISCUSSION

The 1,3-dimethylbarbituric acid 1 on nitration with fuming nitric acid at <40 ^{0}C to yield 1,3- 2,4,6-pyrimidinetrione 2 (Subramania' 1994), which on reduction with tin in the presence of hydrochloric acid at heating for 30 min. gave 5-amino-1,3-dimethyl- hexahydro-2,4,6-pyrimidinetrione 3 (Subramania' 1994). The compound 3 on reaction with corresponding aryl aldehyde, in the presence of acetic acid at reflux for 3 h, furnished the corresponding 1,3- 1-anylmethylidene]aminohexahydro-2,4,6-pyrimidinetrione derivative 4(a-j) in good yields. The corresponding compound 4(a-j) when reacted with thioglycolic acid, in the presence of $ZnCl_2$ in DMF at reflux temperature for 8 h, afforded the corresponding 1,3-dimethyl-5-(4-oxo-2-aryl-1,3-thiazolan-3-yl)hexahydro-2,4,6-pyrimidinetrione 5(a-j) in good yields (Scheme 1).

The structures of compounds were analyzed by their spectral data. IR spectrum of **2,** the absorption bands due to C=O and N-CH_3 are appeared at 1741, 1621 and 2765 cm^{-1}. The NO_2 absorption bands appeared at 1587 and 1372 cm^{-1}. Its ^{1}H NMR spectra, protons of both methyl groups were appeared as a singlet at δ 2.97 and methyne proton attached to NO_2 group appeared as singlet at δ 5.92 ppm Its ^{13}C NMR specta, the signals of barbituric acid ring appeared at δ 82.1, 149.7, 162.3 ppm, the methyl carbon appeared at δ 22.9 ppm. In the IR spectrum of **3,** the absorption bands appeared at 3366 (-NH_2), 1739, 1622 (C=O) and 2762 (CH-NH_2) cm^{-1}. Its ^{1}H NMR spectrum, the signals appeared for the protons of methyl group at δ 2.87, amine group at δ 3.23, CH-NH_2 proton at δ 4.12 ppm. Its ^{13}C NMR specta, the signals appeared for barbituric acid ring at δ 54.3, 152.3, 169.8 and for methyl group at δ 22.7 ppm. The IR spectrum of **4a,** the absorption bands appeared at 2267 (C=N), 1741, 1618 (C=O) cm^{-1}. Its ^{1}H NMR spectrum, the signals appeared for the protons of methyl group at δ 2.87, the proton of barbituric acid (CH-N) and imine (CH=N) appeared as a singlets at δ 4.92 and 7.76 ppm respectively, aromatic protons in the range of δ 7.00-7.05 and 7.62 ppm. Its ^{13}C NMR specta, the signals appeared for barbituric acid at δ 66.1, 152.3, 178.1. The IR spectra of compounds **5a**, C=O absorption bands of barbituric acid and thiazolidinone ring appeared at 1745, 1611 and 1709 cm^{-1} respectively. Its proton NMR spectra, showed the signals for protons of thiazolidinone ring at δ 4.17 (CH_2CO), 5.69 (CH-S) ppm, the other protons appeared at the expected region. Its ^{13}C NMR spectra, showed the signals of barbituric acid at δ 152.7, 167.9, 51.6, the thiazolidinone ring at δ 170.5, 67.2, 35.4 ppm. The other signals were observed at the expected region.

Antibacterial Activity: The *in vitro* antibacterial activity of compounds 5(a-j) were evaluated against Gram +ve bacteria (*B. subtilis*, *B. sphaericus, S. aureus*) and Gram -ve bacteria (*P. aeruginosa*, *K. aerogenes* and *C. violaceum*) by broth dilution method (Villanova, 1982). The bacteria were grown overnight in Luria Bertani (LB) broth at 37°C, harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO.

Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 100 to 0.8 μg/mL. Ten microliters of the broth containing about 10^5 colony-forming units (cfu)/mL of test bacteria wasere added to each well of a 96-well microtiter plate. Culture plates were incubated for 24 h at 37 °C, and the growth was monitored by visually and spectrophotometrically. Penicillin and Streptomycin were also screened under identical conditions for comparison. The minimal inhibitory concentration (MIC, μg/mL) of the compounds 5(a-j) are presented in Table 1.

Antibacterial evaluation of compounds 5(a-j) indicates, that these compounds showed considerable inhibition towards all the tested bacteria. Amongst them, compounds containing 4-hydroxyphenyl (5c), 2,6-difluorophenyl (5g) and 4-hydroxy-3-methoxyphenyl (5i) groups on thizolidionone ring showed considerable activity against tested bacterial strains. Compound, contain 4-chlorophenyl group (5c) and 4-*N*,*N*-dimethylaminophenyl (5h) showed better activity against Gram-positive (except *B. Subtilis)* and Gram-negative bacteria (except *C. Violaceum)*. Similarly, compounds containing 4-methylphenyl (5b), 3-nitrophenyl (5f) and pipernyl (5j) were active against *B. sphaericus, and S. Aureus, respectively*. The other compounds also exhibited considerable antibacterial activities.

Antifungal Activity: The antifungal activity of the compounds 5(a-j) were evaluated against *C. albicans*, *A. fumigates*, *T. rubrum and T. metagrophytes,* using broth dilution method (Villanova, 1982). *C. albicons* was grown for 48h at 28^{o}C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus, T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 ^{o}C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculums size of 10^5 spores/mL. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range 100 to 0.8 µg/mL. Ten micro liters of the both containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/mL of test fungi was added to each well of 96-well microtiter plate. Culture plates were incubated for ~48-72 h at 28 ^{o}C. The antifungal activity of each compound was compared with the standard drug amphotericin B and presented in Table 2. The antifungal evaluation of 5(a-j) indicates that the compounds 5e and 5i which contain 4-hydroxyphenyl and 4-hydroxy-3-methoxyphenyl respectively showed considerable activity against all the fungal strains. The remaining compounds also exhibited significant antifungal activity.

CONCLUSION

A series of new thiazolidinones with barbituric acid scaffolds 5(a-j) were synthesized and evaluated for their antimicrobial activity against various bacteria and fungal strains. The antibacterial activity of compounds containing 4-hydroxyphenyl (5c), 2,6-difluorophenyl (5g) and 4-hydroxy-3-methoxyphenyl (5i) groups on thizolidionone ring showed considerable activity against tested bacterial strains. The antifungal evaluation indicates that the compounds 5e and 5i which contain 4-hydroxyphenyl and 4-hydroxy-3-methoxyphenyl respectively showed considerable activity against all the fungal strains. The other compounds also showed appreciable activity against the test bacteria, fungal strains and emerged as potential molecules for further development.

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