Evaluation of Antimicrobial Activity of some newly Synthesized AZO Compounds Derived from Thiobarbituric Acid

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Abstract: Aim of the present study was to evaluate their antibacterial activity of newly synthesized Azo derivatives of thiobarbituric acid. The compounds 3(a-g) of 5-azoaryl thiobarbiturates dyes were prepared by the coupling of 2-thiobarbituric acid with derivatives diazonium salts, which diazotized of aniline derivatives were carried out in presence of sodium nitrite and mineral acid. All the synthesized compounds were analysed by elemental analysis, IR and ^{1}H NMR, and evaluated in-vitro for their preliminary antibacterial activity against various bacterial such as Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii and Escherichia coli. Antibacterial activity of each compound was compared with standard drug, Gentamicin. The results revealed that the compound, 3g was found to exhibit to good antibacterial activity against both gram (+) and gram (-) bacteria, whereas compounds, 3(a-h) showed moderate antibacterial activity.

Key words: Thiobarbituric acid (TBA), Sulphanilamide, aniline derivative, diazotization, antibacterial activity, antioxidant activity

INTRODUCTION AND OBJECTIVE

2-Thiobarbituric acid (TBA) is structurally as well as synthetically a cyclic malonyl thiourea derivative which contains thiocarbonyl group at second position of pyrimidine 4, 6 dione. It is an intermediate synthetic precursor, from which various thiobarbiturates have been synthesized and have been reported to posses different biological activities such as, antimicrobial,[1] antifungal,[2] antioxidant,[3] antidepressant,[4]anti-tubercular,[5]and anti-convulsant[6].Sulphonamide derivatives are well known pharmacophore since this group has been the main part of the most of the drug structures due to stability and tolerance in human beings. Azo compounds are the largest class of industrially synthesized organic dyes due to their versatile application in various fields, such as dyeing textile fiber, biomedical studies, and advanced application in organic synthesis and high technology areas such as laser, liquid crystalline displays, and electro optical devices. The azo dye sulphonamide of antibacterial drug such as prontosil was the first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infection in humans. In view of the above mention, it was thought of interest to incorporate aryl azo group at C-5 position of 2-Thiobarbituric acid in one molecular framework and to investigate the effect of compounds against antimicrobial and antioxidant activities.

EXPERIMENTAL SECTION

Synthesis of (E)-5-(substituted phenyl diazenyl)-2-thioxodihydropyrimidine-4, 6(1H, 5H)-dione[7] (3a-g)

A cold solution of 2.5 mL of sodium nitrite was added drop wise to ice cold solution of ten different individual substituted aromatic amine in conc. HCl and water. The temperature of the reaction was maintained up to 0-5 ^{0}C during addition. When addition was completed, the solution was kept for 5 minutes with occasional stirring to complete the diazotization. Then it was poured into an ice cold solution of 2-thiobarbituric acid in 10% (20 mL) sodium hydroxide solution. The reaction mixture was allowed to stand in ice bath for 10-15 minutes. The colour products obtained were filtered, washed with water and finally dried. The entire product individually was recrystallized from 50% ethanol.

ANTIMICROBIAL ACTIVITY

Antibacterial activity test by agar-well diffusion method

Antibacterial activities of the seven different compounds were done by agar-well diffusion method[8]. Antibacterial activities were evaluated by measuring the diameter of zones of inhibition. Gentamicin 10µg/mL for gram +ve and gram -ve bacteria, in aliquots of 100 µL were used as reference-controls, for all dyes. Dyes causing the zone of inhibition of 20 mm or more were considered highly active and that having a zone of inhibition less than 20 mm was considered moderately active. Gentamicin 10µg/mL with an average size of zone of inhibition of 20 mm and no zone of inhibition by DMSO 10% solution were taken as reference controls.

DETERMINATIONS OF MIC AND MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active dyes were determined as described by the reported method[13].

RESULTS AND DISCUSSION

Chemistry

A series of 5-(substituted phenyl diazenyl)-2-thioxodihydropyrimidine-4, 6(1H, 5H)-dione (3a-g) were synthesized by coupling of diazonium salt of aniline derivatives/ sulphanilamide 2(a-g) with 2-thiobarbituric acid in presence of sodium hydroxide (scheme). The coupling mechanism of organic reaction was mentioned in the scheme. The crude products were recrystallized from 50% ethanol while some other compounds were recrystallized from mixture of ethanol and DMF. Thiobarbituric acid contains active methylene group at C_5 position which on attacked by strong N_2^+electrophiles to produce Azo thiobarbiturate. The compounds were interpreted by IR, and ^{1}HNMR. In the IR spectra of compounds **3a-g**, the disappearance the absorption band CH_2 stretching at 2720cm^{-1}and the shows of significant stretching vibration bands due to N-H, C=O, N-C=S and N=N were indicated at about 3450–3120 cm^{-1},1705–1700cm^{-1},1455-1415cm^{-1} and 1565-1555 cm^{-1}respectively, and was confirmed that the formation of 3-azo aryl substituted thiobarbiturate by diazotization reaction followed by coupling with different substituted aniline. In all the products were clearly disappeared CH_2 absorption bands in IR due to substituted of N=N group at C-5 of thiobarbituric acid. All the products shows characteristic strong absorption band C=S functional group at 1,320-1,300 cm^{-1}which are respect to cyclic thioamide. The IR spectra of 3g showed three different bands at 3089-3034 cm^{-1}, 1340 cm^{-1} and 1160 cm^{-1} indicate the presence of N-H and SO_2 groups of asymmetrical and symmetrical str respectively. In addition to above IR absorption bands, the product 3b showed most significant bands at 1560-1540, and 1385-1350cm^{-1} with respect to asymmetric and symmetrical stretching of nitro functional group.

ANTIMICROBIAL ACTIVITY

In vitro antibacterial activity of the synthesized compounds was performed against Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii and Escherichia coli using Gentamicin as a reference standard drug. The activity potentials were qualitatively assessed by the presence or absence of inhibition zones, zone diameters, MIC and MBC values. The results of antibacterial activity of the tested compounds (3a-g) were summarized in Tables-2**.** The synthesized compound, 4-[(4, 6-dioxo-2-thioxohexahydropyrimidin-5-yl) diazenyl] benzene sulphonamide was tremendously inhibited Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii and Escherichia coli. The compound, 3g showed greater antibacterial potential against all bacterial strains. The maximum inhibition zones (mm) produced by 3g against Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii and Escherichia coli were 23, 25, 22 and 23 respectively. The compounds 3a to 3f were found to moderate activity against all bacterial stains. The values of the MIC and MBC against micro-organisms were expressed in Table 3. The results showed significant inhibitory effects, with the majority of the compounds with the MIC values 3.41-9.63 mg/mL. These results indicated that the effect of electron withdrawing groups such as nitro, chloro, bromo and special group like sulphomoyl substituted phenyl in TBA ring systems as in basic structural constituents in the synthesized compounds.

CONCLUSION

Present research work involves synthesis of some 5-thiobarbiturate derivatives to explore their antimicrobial activity. The compound (3g) exhibited good antibacterial activity against all bacterial strains but very active against Staphylococcus aureus due to presence sulphonamide and N=N groups in same molecular structural frame.

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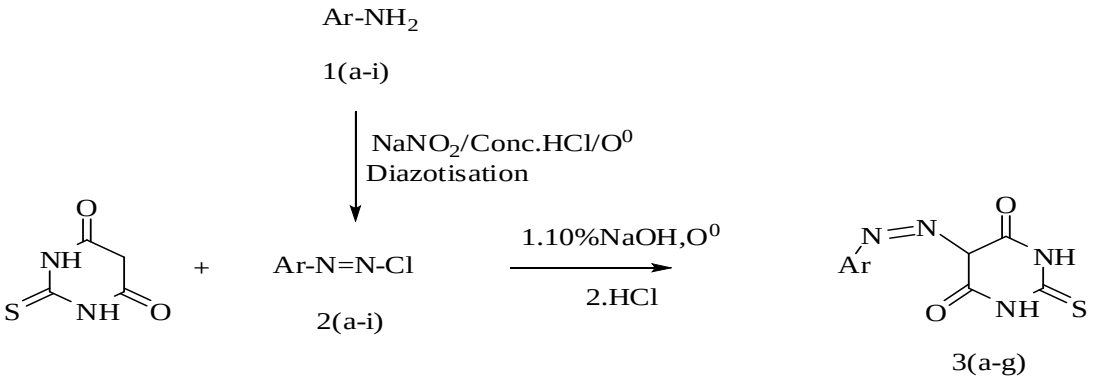

Ar;phenyl,4-nitro phenyl,,4-chloro phenyl,4-methoxy phenyl, 4-bromophenyl, napthyl.4-sulphomoyl phenyl

Table-1 Zone of inhibition in mm of synthesized compounds (3a-g)

Compounds	Bacterial strains			
	Enterococcus faecalis	Staphylococcus aureus	Acinetobacter baumannii	Escherichia coli
3a	20	22	18	16
3b	21	22	20	18
3c	22	24	20	22
3d	20	23	18	19
3e	20	21	21	18
3f	19	22	17	15
3g	23	25	22	23
Gentamicin (10µl/ml)	27	26	25	26
DMSO	-	-	-	-

Table-2.MIC and MBC of synthesized compounds (3a-g)

Compounds		Bacterial strains			
		Enterococcus faecalis	Staphylococcus aureus	Acinetobacter baumannii	Escherichia coli
3a	MIC	4.27	3.41	4.27	9.63
	MBC	9.63	4.27	9.63	21.67
3b	MIC	3.41	3.41	4.27	4.27
	MBC	4.27	4.27	9.63	9.63
3c	MIC	3.41	1.51	4.27	3.41
	MBC	4.27	3.41	9.63	4.27
3d	MIC	4.27	1.51	4.27	4.27
	MBC	9.63	3.41	9.63	9.63
3e	MIC	4.27	3.41	3.41	4.27
	MBC	9.63	4.27	4.27	9.63
3f	MIC	4.27	3.41	9.63	9.63
	MBC	9.63	4.27	21.67	21.67
3g	MIC	3.41	1.51	4.27	3.41
	MBC	4.27	1.51	9.63	4.27