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Synthesis and *In-vitro* Antimicrobial Activity of Pyrimidine Derivatives

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ABSTRACT

In the present work an easy access to a series of pyrimidines bearing a pyronyl side chain in the 4-position is described. These unreported compounds are obtained starting from commercially available dehydroacetic acid (DHA), aromatic aldehydes and S-benzylisothiouronium chloride (SBT). A series of compounds (3a-j) were prepared and tested for their antimicrobial activity. These synthesized compounds showed mild activity against gram +ve bacteria and very less activity against gram -ve bacteria. Compounds 3b, 3i, 3j showed mild activity against gram +ve *Bacillus subtilis* bacteria while against gram -ve *Pseudomonas aeruginosa* compound 3i showed minor activity.

Keywords: Pyrimidine derivatives, Synthesis, Zone of inhibition, Antimicrobial activity.

INTRODUCTION

Pyrimidines represent an important class of heterocycles and their structural framework is not only a key constituent of nucleic bases, alkaloids, and numerous pharmacophores with variety of potent biological activities. Pyrimidines occupy a distinct and unique place in medicine, large array of pyrimidine non-nucleoside derivatives possess a variety of pharmacological properties. These properties include anticancer [1-4], antiviral [5-10], antibacterial [11-13], antifungal [14-16], antiprotozoal [17-21], antihypertensive [22-23], antihistaminic [24], anti-inflammatory [25-26] and central nervous activities. [27-28] Moreover, Novel 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-(substitutedimino) pyrimidine derivatives were reported to possess antiviral, anti-tubercular and antibacterial activity. [29]

Efforts to discover new biologically active compounds with limited side effects in these series are reflected by the still important number of publications devoted to the subject. Due to these unique properties, developments of synthetic methods using different substrates and reagents, which enable rapid access to pyrimidines, are desirable. In most cases, synthesis of pyrimidines is based on the classical condensation reactions between C-C-C and N-C-N components or cross coupling reactions. In continuation to our interest in the chemical and pharmacological properties of pyrimidine derivatives we herein report a new series of 6-substituted-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine derivatives.

MATERIALS AND METHODS

Synthesis of 6-Substituted-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzyl thiopyrimidine

All the reagents and chemicals procured from S.D fine laboratory. 6-substituted-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine (**3**) was synthesized by condensation of 3-cinnmoyl-4-hydroxy-6-methyl-2-oxo-2*H*-pyran (DHA Chalcone) **(2)** with S-Benzylisothiouronium chloride (SBT) in the presence of piperidine as a base and alcohol used as solvent. Since the compound expected from the condensation of SBT with chalcones would be a dihydropyrimidine, it was apparent that oxidation of this dihydropyrimidines had occurred as reported by Dodson and Seyler. [30] The 3-cinnmoyl-4-hydroxy-6-methyl-2-oxo-2*H*-pyran (DHA Chalcone, **2**) was prepared by refluxing DHA (dehydroacetic acid, **1**) with aromatic aldehydes in chloroform and in the presence of base piperidine.

The newly synthesized heterocycles 6-aryl-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine (3a-j) were characterized by IR, ^{1}H-NMR, mass spectral data and elemental analysis. In the IR spectra of compounds, the sharp absorption at 1712–1698 cm^{-1} indicates the existence of the carbonyl group. While analyzing the PMR spectra of compounds, an interesting observation was made. The C_5-H signal which usually appears at about δ 7.3-7.6 in such pyrimidine derivatives appeared at δ 8.8-9.1. Melting points were taken on Lab India melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument using TMS as an internal standard. IR spectra were recorded on a Perkin Elmer-2400 spectrophotometer.

of substituted 3-cinnamoyl-4-hydroxy-6-methyl-2-oxo-2*H*-pyran (Chalcone analogues of DHA)

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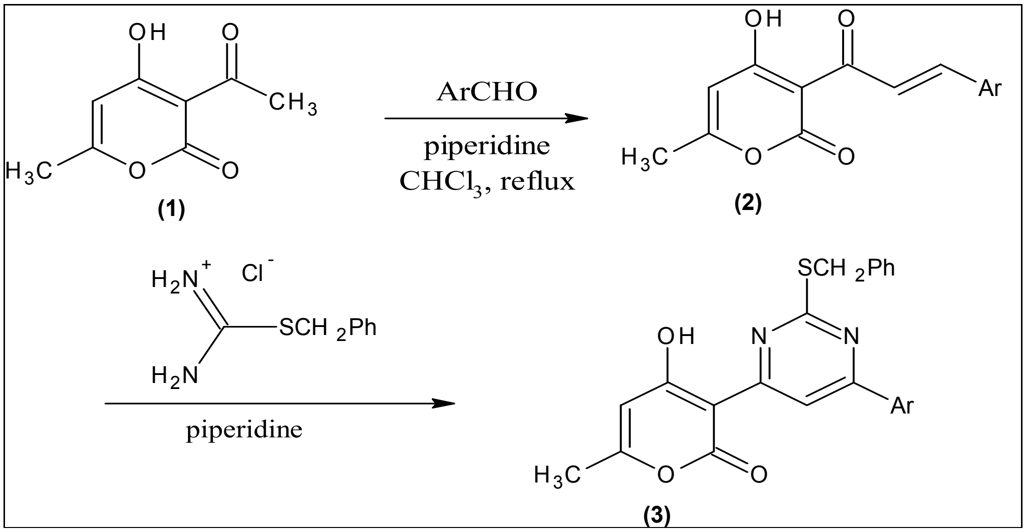
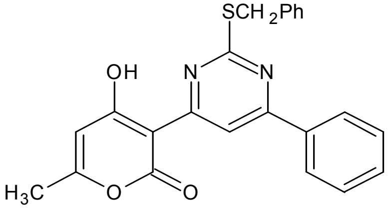


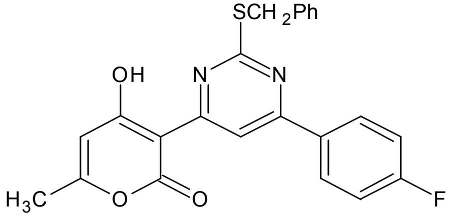
Scheme

6-Phenyl-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine [3a]



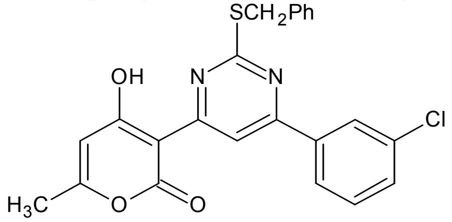
M.p. 214-216°C , yield 35%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.29(s, 3H, CH_3), 4.62 (s, 2H, CH_2), 5.99 (s, 1H, $C_{5'}$-H, DHA), 8.95 (s, 1H, C_5-H), 7.28-8.23 (m, 10H, arom.)
IR (ν_{max}, KBr): 1698 cm^{-1}(C=O), 1583, 15233, 1456 cm^{-1}(C=C arom.), 2984 cm^{-1} (HC=CH str.)

6-(4-Fluorophenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3b]



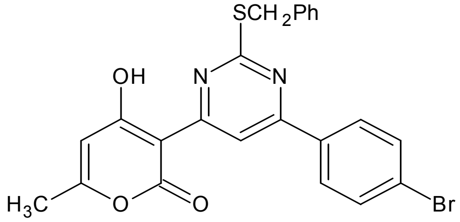
M.p. 255-257°C , yield 35%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.30(s, 3H, CH_3), 4.59 (s, 2H, CH_2), 5.98 (s, 1H, $C_{5'}$-H, DHA), 8.89 (s, 1H, C_5-H), 7.1-8.2 (m, 9H, arom.)
IR (ν_{max}, KBr): 1698 cm^{-1}(C=O), 1579, 1533 cm^{-1} (C=C arom.)

6-(3-Chlorophenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3c]



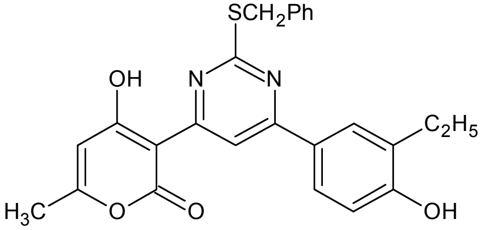
M.p. 185-187°C , yield 30%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.3(s, 3H, CH_3), 4.59 (s, 2H, CH_2), 5.99 (s, 1H, $C_{5'}$-H, DHA), 8.9 (s, 1H, C_5-H), 7.2-8.1 (m, 9H, arom.)
IR (ν_{max}, KBr): 1705 cm^{-1}(C=O), 1558, 1520 cm^{-1}(C=C arom.)

6-(4-Bromophenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3d]



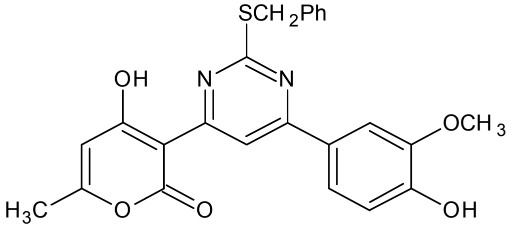
M.p. 245-247°C , yield 33%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.30 (s, 3H, CH_3), 4.58 (s, 2H, CH_2), 5.99 (s, 1H, $C_{5'}$-H, DHA), 8.91 (s, 1H, C_5-H), 7.648-7.677 (d, 2H, *J*=8.7Hz, Ar-H), 8.049-8.078 (d, 2H, *J*=8.7Hz, Ar-H), 7.28-7.56 (m, 5H, arom.)
IR (ν_{max}, KBr): 1697 cm^{-1}(C=O), 1585, 1530, 1462 cm^{-1}(C=C arom.)

6-(4-Hydroxy-3-ethylphenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3e]



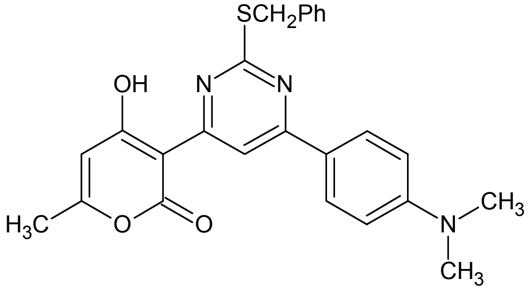
M.p. 232-235°C , yield 35%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.28(s, 3H, CH_3), 4.59 (s, 2H, CH_2), 5.95 (s, 1H, $C_{5'}$-H, DHA), 8.85 (s, 1H, C_5-H), 6.9-7.8 (m, 8H, arom.)
IR (V_{max}, KBr): 1690 cm^{-1}(C=O), 1566, 1528, 1412 cm^{-1}(C=C arom.), 3217 cm^{-1} (O-H str.), 2986 cm^{-1} (HC=CH str.)

6-(3-Methoxy-4-hydroxyphenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3f]



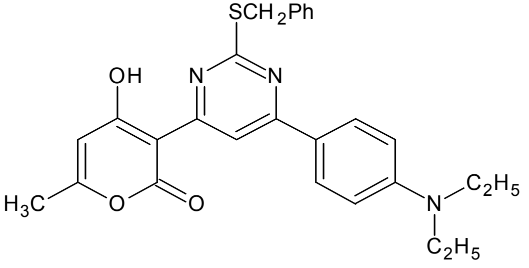
M.p. 227-229°C , yield 33%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.29 (s, 3H, CH_3), 4.6 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3) 5.9 (s, 1H, $C_{5'}$-H, DHA), 8.86 (s, 1H, C_5-H), 6.9-7.8 (m, 8H, arom.)
IR (ν_{max}, KBr): 1690 cm^{-1}(C=O), 1566, 1458 cm^{-1}(C=C arom.), 3194 cm^{-1} (O-H)

6-(4-Dimethylaminophenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine [3g]



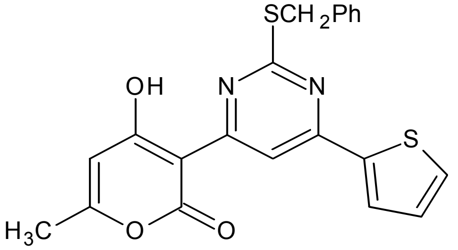
M.p. 253-255°C , yield 30%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.26 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 3.1 (s, 6H, CH_3), 5.9 (s, 1H, $C_{5'}$-H, DHA), 8.8 (s, 1H, C_5-H), 6.76-6.79 (d, 2H, *J*=9Hz, Ar-H), 8.161-8.190 (d, 2H, *J*=8.7Hz, Ar-H), 7.2-7.6 (m, 5H, arom.)
IR (ν_{max}, KBr): 1697 cm^{-1}(C=O), 1582, 1535, 1474 cm^{-1}(C=C arom.), 2916 cm^{-1} (HC=CH str.)

6-(4-Diethylaminophenyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzyl thiopyrimidine [3h]



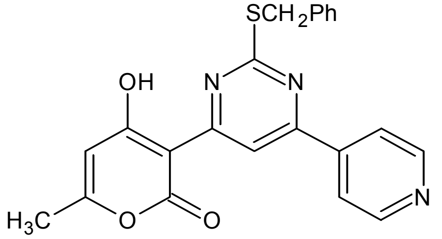
M.p. 258-260°C , yield 34%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.25 (S,3H, CH_3), 4.62 (s, 2H, CH_2), 5.91 (s, 1H, $C_{5'}$-H, DHA), 8.78 (s, 1H, C_5-H), 6.732-6.762 (d, 2H, *J*=9Hz, Ar-H), 8.143-8.173 (d, 2H, *J*=9Hz, Ar-H), 7.2-7.6 (m, 5H, arom.)
IR (ν_{max}, KBr): 1697 cm^{-1}(C=O), 1574, 1528, 1458 cm^{-1}(C=C arom.)

6-(2-Thiophene)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine [3i]



M.p. 215-217°C , yield 36%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.28 (s, 3H, CH_3), 4.57 (s, 2H, CH_2), 5.95 (s, 1H, $C_{5'}$-H, DHA), 8.7 (s, 1H, C_5-H), 7.2-7.9 (m, 8H, arom.)
IR (ν_{max}, KBr): 1698 cm^{-1}(C=O), 1579, 1525, 1428 cm^{-1}(C=C arom.), 2924 cm^{-1} (HC=CH str.)

6-(4-Pyridyl)-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthio pyrimidine [3j]



M.p. 254-256°C , yield 30%
^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.32 (s, 3H, CH_3), 4.59 (s, 2H, CH_2), 6.02 (s, 1H, $C_{5'}$-H, DHA), 9.0 (s, 1H, C_5-H), 6.7-8.1 (m, 9H, arom.)
IR (ν_{max}, KBr): 1700 cm^{-1}(C=O), 1580, 1533, 1454 cm^{-1}(C=C arom.), 2924 cm^{-1} (C-H str.)

Comp. No.	Ar	Yields (%)	M.p.(ºC)	Mol. Formula
3a	Ph	35	214-16	$C_{20}H_{16}O_3N_3S_2$
3b	4-FC_6H_4	32	255-57	$C_{20}H_{16}O_3N_3S_2$
3c	3-ClC_6H_4	35	185-87	$C_{23}H_{17}O_3N_2SCl$
3d	4-BrC_6H_4	31	245-47	$C_{23}H_{17}O_3N_2SBr$
3e	4-OH-3-Et- C_6H_3	30	232-35	$C_{25}H_{22}O_4N_3S$
3f	3-OCH_3-4-OH- C_6H_3	33	227-29	$C_{24}H_{20}O_5N_2S$
3g	$(CH_3)_2NC_6H_4$	30	253-55	$C_{25}H_{23}O_3N_3S$
3h	$(C_2H_5)_2NC_6H_4$	38	258-60	$C_{25}H_{27}O_3N_3S$
3i	2-Thienyl	25	215-17	$C_{20}H_{16}O_3N_3S_2$
3j	4-Pyridyl	35	254-56S	$C_{22}H_{17}O_3N_3S$

Fig. 1: Melting point and respective % yield of different derivative

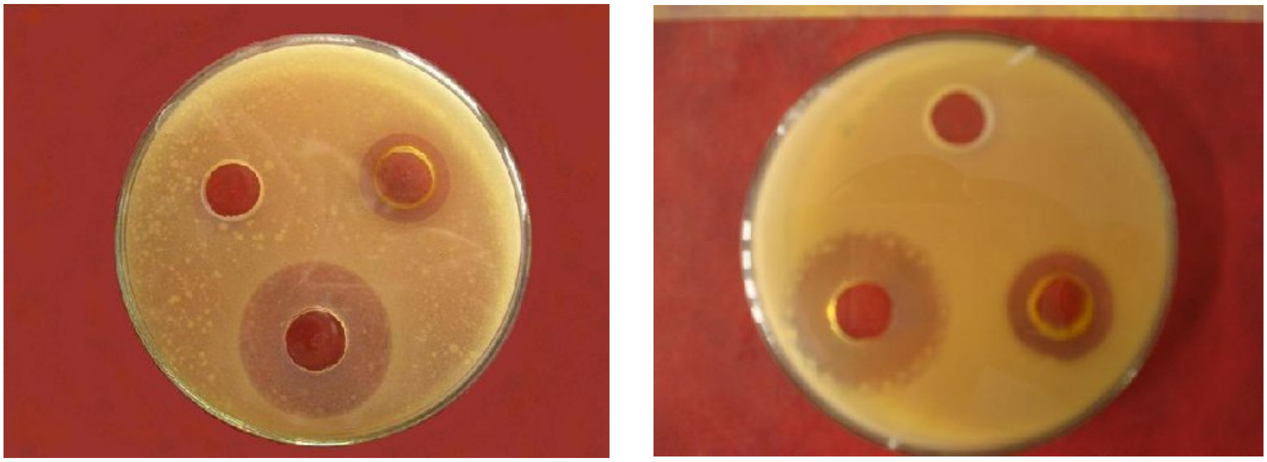


Fig. 2: Zone of inhibition of test compounds against gram+ve bacteria (*Bacillus subtilis*)

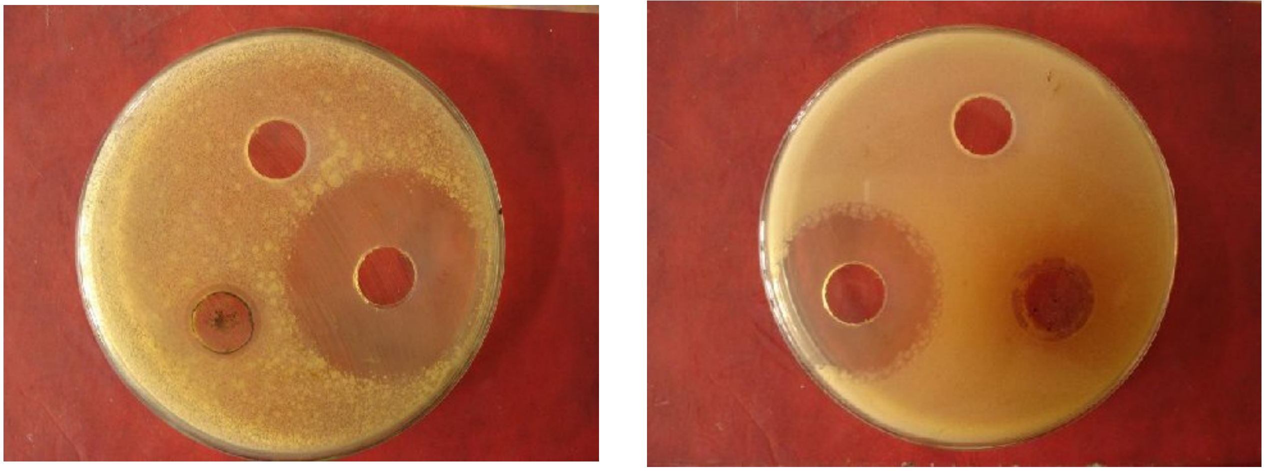


Fig. 3: Zone of inhibition of test compounds against gram-ve bacteria (*Pseudomonas aeruginosa*)

Table 1: Zone of inhibition of ciprofloxacin (10µg/ml) against *Bacillus subtilis* was 22 mm

Sample	10µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml	750µg/ml
3a	-	-	-	-	3mm	7mm
3b	-	-	-	4mm	7mm	12mm
3c	-	-	-	-	-	-
3d	-	-	-	-	-	-
3e	-	-	-	-	-	3mm
3f	-	-	-	-	-	-
3g	-	-	-	-	4mm	6mm
3h	-	-	-	-	2mm	5mm
3i	-	-	-	6mm	8mm	11mm
3j	-	-	-	3mm	5mm	9mm

Table 2: Zone of inhibition of ciprofloxacin (10µg/ml) against *Pseudomonas aeruginosa* was 30 mm

Sample	10µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml	750µg/ml
3a	-	-	-	-	3mm	6mm
3b	-	-	-	-	4mm	7mm
3c	-	-	-	-	-	-
3d	-	-	-	-	-	-
3e	-	-	-	-	-	-
3f	-	-	-	-	-	-
3g	-	-	-	-	-	4mm
3h	-	-	-	-	-	-
3i	-	-	-	2mm	5mm	9mm
3j	-	-	-	-	-	5mm

A solution of 3.36g (**1**, 0.02mol) of dehydroacetic acid, 10 drops of piperidine and 0.02 mol of the aldehyde in 25 ml of chloroform were refluxed for 8-10 hours. Ten ml of the chloroform-water azeotrope was separated in Barrett-type distilling receiver. Crystals of the product, which separated on slow evaporation of the remaining chloroform, were collected and recrystallized

Preparation of 6-aryl-4-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-2-S-benzylthiopyrimidine

Chalcone analogue of DHA (0.01mmol) was dissolved in alcohol and to the resulted solution was added SBT (0.01mmol) and 4-5 drops of piperidine and the resulting solution was refluxed for 3-4 hours. The excess of solvent was evaporated and the crystals were collected.

Antimicrobial activity

Sterilization

First step to sterilize the glass apparatus and other equipments. Glass apparatus are sterilized using hot air oven while cotton gloves are sterilized by using moist air sterilization in autoclave.

Preparation of Culture Medium

Then the culture medium is prepared. The culture medium used was Nutrient Agar Medium. Composition of Nutrient Agar is as follows:

Beef Extract	-	10.0g	Peptone	-	10.0g
Sodium Chloride	-	5.0g	Distilled Water	-	1000 ml
Agar	-	15.0g			

Weigh accurately the ingredients and dissolve them with the aid of heat till a homogeneous solution is obtained. Adjust to pH 8.0 to 8.5 using 5M sodium hydroxide. Filter if necessary. In some amount of hot water agar is added with continuous stirring. Then add this to the nutrient broth and mixed properly. Then sterilize it by autoclaving at 115°C for 30 minutes.

Agar Well Diffusion Method

Test solutions of each sample were prepared at a concentration of 10, 50, 100, 250, 500 and 750µg/ml. Ciprofloxacin was taken as standard for antibacterial activity at a concentration of 10µg/ml. Specified media were prepared and sterilized by an autoclave. For antibacterial activity, the media was inoculated with the test organisms (one day old subculture) by uniform mixing and was poured into sterile Petri dishes to a uniform depth and then allowed to cool and solidify at room temperature in an aseptic room. This provided the uniform surface for the growth of bacterium and was used for antibacterial activity studies. For antifungal activity, spread plate method was employed. Then the wells were made in solidified media with the help of sterile glass borer of 8 mm diameter in such a way that there was no overlapping of zone of inhibition. Plates were kept at room temperature for half an hour for diffusion of the sample into agar media. The organism-inoculated Petri dishes were incubated at specified temperature for specified time. After the incubation period was over, the zones of inhibition produced by the sample in different plates were measured and recorded immediately. The same procedure was done in triplicate.

RESULTS

The anti-bacterial activity of the synthesized compounds shows mild activity against gram +ve bacteria and negligible activity against gram –ve bacteria.

Compounds 3b, 3i, 3j showed mild activity against gram +ve *Bacillus subtilis* bacteria while against gram -ve *Pseudomonas aeruginosa* compound 3i showed minor activity.

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