Synthesis and antimicrobial activity of novel oxime derivatives of phenothiazine

Ashutosh Barvea, *, Malleshappa Noolvi1, Niharika Subhedara, Vishnu Dev Gupta2 and Gaurav Bhatiag

a Department of Pharmaceutical Chemistry, Bhanwar Nahata College of Pharmacy, Mandasaur, 458001, India
b Department of Pharmaceutical Chemistry, Amar Shaheed Baba Aji Singh Jujhar Singh Memorial College of Pharmacy, Bhopal, 462001, India
c Department of Pharmacognosy, Shriram College of Pharmaceutical Education, Indore, 452001, India

*Corresponding author at: Department of Pharmaceutical Chemistry, Bhanwar Nahata College of Pharmacy, Mandasaur, 458001, India. Tel: +91.9893107417; fax: +91.1881263655. E-mail address: ashutoshbarve@gmail.com (A. Barve).

ARTICLE INFORMATION
Received: XX November 2010
Received in revised form: XX January 2011
Accepted: XX January 2011
Online: 30 September 2011

KEYWORDS
Coumarin
Oxime
Phenothiazine
Antimicrobial activity
Anti-bacterial activity
Anti-fungal activity

ABSTRACT
A series of 4-methyl-2-oxo-pyryl-phenothiazines (IIa-j) followed by 4-methylpyrano-(2,3-β)-phenothiazine-2(11H)-one oxime (IVa-j) were synthesized by using 7-hydroxy-4-methylcoumarin (I). Further reaction of (I) was carried out with substituted aromatic amines (a-j) to convert into 7-arylamino-4-methyl-coumarin (IIa-j). Additionally (IIa-j) was treated with sulphur in presence of iodine to obtain a series of novel 4-methyl-2-oxo-pyryl-phenothiazine (IIIa-j) derivatives, which on treatment with hydroxylamine hydrochloride afforded the title compounds i.e. 4-methylpyrano-(2,3-β)-phenothiazine-2(11H)-one oxime (IVa-j). The structures of these compounds were confirmed by IR, NMR and Mass spectral analysis. The newly synthesized compounds were evaluated for antibacterial and antifungal activity. The results show that compound IIIa, IIIc, IIIh, IIIl, IVa, IVI and IVj exhibited moderate to good antibacterial and antifungal activity at 5-100 mcg/mL.

1. Introduction
In recent times microbial resistance against antimicrobial agents has increased remarkably. New prototype compounds are required to deal with this problem, so discovery of novel synthetic and semi-synthetic product as antimicrobials is the prerequisite of present health scenario, and continuous effort in development of same is very much required. Pharmacological properties of coumarin aroused our interest to explore new analogs of coumarin for antimicrobial activity. Coumarin and its analogues are widely distributed in plants and they are responsible for wide variety of the activities especially antibacterial and antifungal [1-10]. The in vitro antimicrobial activity of phenothiazine was first described by Paul Ehlich early in the twentieth century [11]. Various studies show and support those phenothiazines may be used for the management of bacterial or fungal infections [12-21]. The study also shows that phenothiazine not only itself having antimicrobial effect but also it show synergistic interaction with other antimicrobial [22]. Moreover various heterocyclic compounds containing oxime, and the complexes of oximes with different transition metals are reported in the literature and. Found: to be active as antibacterial, antitubercular, antilepil, antiviral and antimalarial [23-34] so we consider these reports and incorporate this functional moiety into our scheme. Therefore the development of facile synthetic routes to achieve access to these molecules is of prime interest. In view of the above mentioned pharmacological applications of phenothiazine coumarin and oxime and in continuation of our research on the synthesis of biologically active molecules, we considered undertaking the design and synthesis of hitherto unknown phenothiazine derivatives. Further, the increasing number of multdrug resistant pathogens has led us to screen the newly synthesized derivatives against the representative panel of Gram-positive [Gr (+)] and Gram-negative [Gr (-)] bacteria and fungi.

2. Experimental
2.1. Instrumentation
Melting points were determined in Thermonik melting point apparatus and are uncorrected. IR spectrum was recorded on Thermonicolet FT-IR 200 spectrophotometer by using KBr pellet values are expressed in cm⁻¹. NMR spectra were recorded in DMSO-d₆, using varian 400 MHz mercury plus and chemical shift are reported in δ (ppm). Mass spectra were recorded on GCM-QP 2010 Shimadzu and mass values are reported in m/z. All the chemicals used are of reagent grade and substituted aromatic amines and other chemicals used during synthesis are of synthesis fine grade.

2.2. Synthesis
The synthesis of 7-hydroxy-4-methylcoumarin (I) was carried out by the reaction of resorcinol and ethylacetocetate; it is well established [35]. The various substituted 7-arylamino-4-methyl-coumarin (IIa-j) were prepared by reacting with substituted aromatic amines (a-j). The 7-arylamino-4-methyl-coumarins (IIa-j) were further converted into 4-methyl-2-oxo-pyranyl-phenothiazines (IIIa-j) by the action of sulphur in presence of iodine. Further these novel 4-methyl-2-oxo-pyranyl-phenothiazines (IIIa-j) derivatives, on treatment with hydroxylamine hydrochloride were converted to 4-
methylpyrano-[2,3-β]-phenothiazine-2(11H)-one oxime (IVa-j) derivatives as shown in Scheme 1. The reaction and purity of compounds were monitored by TLC using precoated silica gel. Structures of the compounds were confirmed by spectral studies.

2.2.1. Preparation of compounds (IIa-j) [35,36]

In 50 mL beaker, mixture of resorcinol (3.7 g) and ethanecarboxylic (4.4 mL) was heated with water and sodium carbonate solution to obtain sodium carbonate (15 mL) at 5 °C with constant stirring for 30 minutes. Mixture was poured out to the crushed ice (about 100 g), with vigorous stirring. 7-hydroxy-4-methyl coumarin (I) was precipitated. Suspension was filtered; crude was dissolved in cold aq sodium hydroxide (10%) solution and reprecipitated it by addition of dilute hydrochloric acid. Crude was decolorized and re-crystallized from coal and ethanol respectively [35]. A mixture of 7-hydroxy-4-methyl coumarin (I) (1 mol) and primary aromatic amine (1 mol) in absolute ethanol (20 mL) was heated under reflux in presence of anhydrous ZnCl2 (0.2 g) for 5 h. It was cooled and the separated crude mass was filtered, washed repeatedly with cold water, dried and recrystallized from methanol, the synthesized 7-arylaminoo-4-methyl-coumarin (IIa-j) and sulphur powder (1.0 g) were heated together at 150-160 °C for 3 h in the presence of iodine (150 mg). The mixture was cooled to room temperature and treated with dilute hydrochloric acid (100 mL) to remove unreacted amine and washed repeatedly with warm water. Residue was dried in vacuum to yield phenothiazine analogs (IIa-j) [36].

4-methylpyrano-[2,3-β]-phenothiazin-2(11H)-one (IIa): Yield: 54.5%. M.p.: 180-182 °C. FT-IR (KBr cm−1): 3446 ν(N-H) (phenol), 3101 ν(C-H) (phenol), 2950 ν(C-H) (phenol), 1738 ν(C=O) (pyraneone), 1571 ν(C-N) (hetero), 1450 ν(C=C), 665 ν(C-S) (hetero). 1H NMR (400 MHz, DMSO-d6, δ, ppm): 6.92 (s, 1H, Ar-H), 6.30-6.80 (m, 4H, Ar-H), 6.26 (s, 1H, Ar-H), 5.67 (s, 1H, CO-CH3), 4.17 (s, 1H, C-NH-C), 1.70 (s, 3H, C-C3). 13C NMR (400 MHz, DMSO-d6, δ, ppm): 160.9 (1C, >C=O), 153.4-110.9 (14C, Ar-C), 151.3-111.5 (14C, Ar-C). MS (DIPMS, m/z): 440.04 (M+) and HMS calculated for C21H15N3OS: 440.046.

10-chloro-4-methylpyrano-[2,3-β]-phenothiazin-2(11H)-one (IIb): Yield: 95.2%. M.p.: 183-185 °C. FT-IR (KBr cm−1): 3446 ν(N-H) (hetero), 3059 ν(C=O) (phenol), 2850 ν(C-H) (carboxyl), 1571 ν(C-N) (hetero), 1450 ν(C-C) (aromatic), 1090 ν(C=O) (pyraneone), 160.3 ν(C=O) (pyraneone), 665 ν(C-S) (hetero). 1H NMR (400 MHz, DMSO-d6, δ, ppm): 6.21-7.10 (m, 5H, Ar-H), 5.74 (s, 1H, CO-CH3), 4.15 (s, 1H, C-NH-C), 1.68 (s, 3H, C-C3). 13C NMR (400 MHz, CDCl3, δ, ppm): 160.8 (1C, >C=O), 152.2-110.3 (14C, Ar-C), 21.4 (CH3 of α-pyranone). MS (DIPMS, m/z): 296.04 (M+) and HMS calculated for C16H11NO3S: 297.046. Found: 297.0456.

Barve et al. / European Journal of Chemistry 2 (3) (2011) 388-393

389
1395 ν(C=O) [acid], 1271 ν(O=H) [acid], 690 ν(C=S) [hetero]. 1H NMR (400 MHz, DMSO-d6, δ, ppm): 10.57 (s, 1H, O=H-COOH), 7.87-7.92 (dd, J=8 Hz, 1H, Ar-H-), 7.04 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.36 (s, 1H, Ar-H), 5.82 (s, 1H, C=H), 4.12 (s, 1H, C=H-C), 1.71 (s, 3H, -C3H7), 1.07 (s, 3H, C-C3H7). 13C NMR (400 MHz, CDCl3, δ, ppm): 168.5 (1C, >C=N=O), 153.1-105.8 (14C, Ar-H), 5.05 (s, 1H, N=C-C=H), 4.17 (s, 1H, C=OH), 1.78 (s, 3H, C3H7). MS (DIPMS, m/z): 305.04 (M+) and HMS calculated for C16H12N2O3S: 312.056. Found: 312.0550.

10-hydroxy-4-methylpyrano[2,3-ß]phenothiazin-2(11H)-one oxime (IVb): Yield: 45.55%. M.p.: 184-186 °C. FT-IR (KBr, cm-1): 3429 ν(O-H) (phenol), 3419 ν(N=H) (hetero), 3315 ν(O=H) (oxime), 3053 ν(C=H) (aliphatic), 2889 ν(C=H) (aliphatic), 1637 ν(C=N) (oxime), 1533 ν(C=N) (hetero), 1438 ν(C=O) (carboxylic). 1H NMR (400 MHz, DMSO-d6, δ, ppm): 6.30-6.72 (m, 5H, Ar-H), 5.18 (s, 1H, N=C-C-H), 5.05 (s, 1H, C=OH), 4.13 (s, 1H, C=NH), 2.03 (s, 1H, C=-N=OH). 13C NMR (400 MHz, CDCl3, δ, ppm): 168.1 (1C, >C=N=O), 153.1-105.8 (14C, Ar-C), 22.3 (1C, -C3H7). MS (DIPMS, m/z): 311.05 (M+) and HMS calculated for C16H12N2O3S: 312.056. Found: 312.0550.

9-hydroxy-4-methylpyrano[2,3-ß]phenothiazin-2(11H)-one oxime (IVc): Yield: 45.4%. M.p.: 194-196 °C. FT-IR (KBr, cm-1): 3384 ν(N=H) (hetero), 3332 ν(O=H) (phenol), 3211 ν(O=H) (oxime), 3053 ν(C=H) (aliphatic), 2889 ν(C=H) (aliphatic), 1637 ν(C=N) (oxime), 1533 ν(C=N) (hetero), 1438 ν(C=O) (carboxylic), 1220 ν(C=O) (carboxylic), 665 ν(C=O) (oxime). 1H NMR (400 MHz, DMSO-d6, δ, ppm): 6.15-6.69 (m, 5H, Ar-H), 5.19 (s, 1H, N=C-C-H), 5.06 (s, 1H, Ar-OH), 4.17 (s, 1H, C=NH), 2.04 (s, 1H, C= N=OH), 1.78 (s, 3H, C3H7). 13C NMR (400 MHz, CDCl3, δ, ppm): 168.5 (1C, >C=N=O), 154.0-103.7 (14C, Ar-C), 22.6 (1C, -

### Scheme 1

2.2.2. Preparation of compounds (IVa-j) [37,38]

To a solution of the keto-ester (1.44 g, 10 mmol) and benzyl-hydroxyamine (1.60 g, 10 mmol) in ethanol (30 mL) was added pyridine (5.0 mL, 62 mmol) in 1 portion. The reaction mixture was heated at 55 °C for 24 h and then concentrated on a rotary evaporator. The residue was partitioned between ether (150 mL) and water (50 mL). The organic layer was sequentially washed with hydrochloric acid (0.5 N, 60 mL) and water (30 mL), and then dried over Magnesium sulphate. Concentration in vacuo provided the oxime (2.50 g, 100%) as a 3:1 mixture of E/Z isomers as a solids or liquid.

4-methylpyrano[2,3-ß]phenothiazin-2(11H)-one oxime (IVA): Yield: 46.6%. M.p.: 186-188 °C. FT-IR (KBr, cm-1): 3396 ν(N=H) (hetero), 3252 ν(O=H) (oxime), 3007 ν(C=H) (aromatic), 2840 ν(C=H) (aliphatic), 1622 ν(C=N) (oxime), 1487 ν(C=N) (hetero), 1412 ν(C=C) (aromatic), 703 ν(C=S) (hetero). 1H NMR (400 MHz, DMSO-d6, δ, ppm): 6.92 (s, 1H, Ar-H), 6.26-6.68 (m, 4H, Ar-H), 6.07 (s, 1H, Ar-H), 5.05 (s, 1H, N=C-C=H), 4.17 (s, 1H, C=N=H-C), 2.07 (s, 1H, C=O), 1.68 (s, 3H, C3H7). 13C NMR (400 MHz, CDCl3, δ, ppm): 168.3 (1C, >C=N=O), 153.3-106.4 (14C, Ar-C), 22.5 (1C, -C3H7). MS (DIPMS, m/z): 295.06 (M+) and HMS calculated for C17H11N2O4S: 296.061. Found: 296.0609.
8-hydroxy-4-methylpyrano[2,3-b]phenothiazin-2(1H)-one oxime ([IVd]: Yield: 43.6%. M.p.: 178-180°C. FT-IR (KBr, cm⁻¹): 3373 v(O-H) (phenol), 3222 v(N-H) (hetero), 3101 v(O-H) (oxide), 3029 v(C-H) (aromatic), 2862 v(C-H) (aliphatic), 1631 v(C=N) (oxide), 1520 v(C=N) (hetero), 1415 v(C=O) (aromatic), 1253 v(C-O) (alcoholic), 700 v(C-S) (hetero). H NMR (400 MHz, DMSO-d₆, δ ppm): 6.36-6.71 (m, 5H, Ar-H), 5.18 (s, 1H, N=C=H), 5.06 (s, 1H, Ar-Oh), 4.17 (s, 1H, C-N-F), 2.10 (s, 3H, C-OH), 1.60 (s, 3H, C-Cl). 13C NMR (400 MHz, CDCl₃, δ ppm): 167.4 (1C, >C=N-OH), 152.5-156.2 (14C, Ar-C), 21.3 (1C, -CH₃). MS (DIPMS, m/z): 311.05 (M⁺) and HMs calculated for C₁₉H₁₈N₂O₂S: 312.056. Found: 312.050.

10-chloro-4-methylpyrano[2,3-b]phenothiazin-2(1H)-one oxime ([IVe]: Yield: 43%. M.p.: 192-194°C. FT-IR (KBr, cm⁻¹): 3338 v(N-H) (hetero), 3255 v(O-H) (oxide), 3049 v(C-H) (aromatic), 2920 v(C-H) (aliphatic), 1653 v(C=N) (oxide), 1591 v(C-N) (hetero), 1499 v(C=C) (aromatic), 1088 v(C-C) (aromatic), 866 v(C-S) (hetero). H NMR (400 MHz, DMSO-d₆, δ ppm): 7.09-7.07 (m, 1H, Ar-H), 6.82-6.84 (d, J=6.8 Hz, 1H, Ar-H), 6.61-6.64 (t, J=6 Hz, 1H, Ar-H), 6.74 (s, 1H, Ar-H), 6.06 (s, 1H, Ar-H), 5.10 (s, 1H, N=C=H), 4.15 (s, 1H, C=N-F), 1.97 (s, 1H, C=O-H), 1.68 (s, 3H, C-CH₃). 13C NMR (400 MHz, CDCl₃, δ ppm): 163.2 (1C, >C=N-OH), 153.6-104.3 (14C, Ar-C), 22.7 (-CH₃). MS (DIPMS, m/z): 329.02 (M⁺) and HMs calculated for C₁₉H₁₇ClN₂O₂: 330.023. Found: 330.022.

2-hydroxymino)-4-methyl-2,11-di-hydroipyranol[2,3-b]phenothiazin-8-carboxylic acid ([IVj]: Yield: 48.3%. M.p.: 168-170°C. FT-IR (KBr, cm⁻¹): 3263 v(N-H) (hetero), 3252 v(O-H) (oxide), 3053 v(C-H) (aromatic), 2894 v(C-H) (aliphatic), 1685 v(C=O) (acid), 1622 v(N=O) (oxide), 1587 v(C-N) (hetero), 1450 v(C-O) (acid), 1402 v(C=C) (aromatic), 1319 v(O-H) (acid). 689 v(C=S) (hetero). H NMR (400 MHz, DMSO-d₆, δ ppm): 10.57 (s, 1H, Ar-COOH), 7.87-7.92 (dd, J=6 Hz, 1J, Ar-H), 6.91 (s, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 6.09 (s, 1H, Ar-H), 5.06 (s, 1H, N=C=H), 1.72 (s, 3H, C-CH₃). 13C NMR (400 MHz, CDCl₃, δ ppm): 168.2 (1C, >C=N-OH), 169.8 (1C, >C=O), 155.8-105.7 (14C, Ar-C), 223 (1C, -CH₃). MS (DIPMS, m/z): 339.05 (M⁺) and HMs calculated for C₂₆H₁₆N₂O₃S: 340.051. Found: 340.0528.

2.3. Biological activity

The synthesized compounds were screened for the antibacterial and antifungal activity by using the agar-cup technique in nutrients agar and potato dextrose agar media, respectively [39-50]. Ciprofloxacin and gresifolin were used as standard drug for the antibacterial and antifungal activity respectively and zone of inhibition of all newly synthesized compounds ([Illa-j]) and ([IVa-j]) was measured against these standard drugs (Table 1 and 2). The novel synthesized compounds have shown moderate activity against bacterial strain compared to standard drug. The title compounds have showed the better antibacterial activity and antifungal activity compared with the standard drug. We used microbial strains - Staphylococcus aureus (NCIM 2602); Bacillus subtilis (NCIM 2413) as GR (+) bacterial strain, Escherichia coli (NCIM 2665) and Pseudomonas aeruginosa (NCIM 5225) GR (-) bacterial strains as Saccharomyces cerevisiae (NCIM 2220); Candida albicans (NCIM 3471); Aspergillus niger (NCIM 813) as fungal strains for biological test.

3. Result and discussion

We have synthesized a series of ten novel 4-methyl-2-oxopyranyl-phenothiazines ([Illa-j]) derivatives and their oxime derivatives i.e. 4-methylpyrano-[2,3-b]-phenothiazine-2(1H)-one oxime ([Iva-j]) using 7-hydroxy-4-methyl-coumarin. Structures of the synthesized compounds were established on the basis of IR, 1H NMR, Mass and HMs spectral data in order to substantiate the structures of the compounds.

Compounds ([Illa-j]) have showed presence of absorption band at 670-715 cm⁻¹, confirmed the formation of phenothiazine ring (C-S hetero stretching), sharp bands at 1715-1775 cm⁻¹ gave conformation of C=O in α-pyrene ring in their respective spectra. Furthermore compounds ([Iva-j]) have showed presence of absorption band ranging from 1620-1690 cm⁻¹ for oxide (C=O) (oxide) and also showed presence of absorption band ranging from 3150-3300 cm⁻¹ for oxide (O=H) (oxide) gave conformation of formation of oxide (C=O) oxide group in their respective spectra.
In particular, it must be pointed out that in $^{1}H$ NMR the characteristic peaks at $\delta$ 1.76 ppm ($CH_3$ group of $\alpha$-pyrone ring), $\delta$ 4.17 ppm ($NH$ group of phenothiazine), $\delta$ 5.67 ppm ($C-H$-Pyran), 6.26 ($C-H$-Ar) and a multiplate at $\delta$ 6.30-6.88 ppm ($4H$, $C-H$-Ar) indicate the presence of above groups in their respective structure.

The compounds (IIa-j) showed prominent singlet at $\delta$ 6.90-9.20 ppm for $\alpha$-pyrone, peak at $\delta$ 6.30-7.20 ppm for aromatic protons.

Compound (IVA-j) have shown presence of a singlet between $\delta$ 9.10-9.05 ppm indicate the formation of oxime ($>C=N-OH$) by simple condensation process in all the spectra has confirmed the formation of oxime derivative and remaining peak for rest of the structure have been observed similar to that of compound (IIa-j).

In $^{13}C$ NMR the characteristic peaks of keto group at 160.90 ppm confirms the carbonyl group at pyranone ring, peaks appeared at 110-152 ppm showed the peaks of aromatic carbon in molecule, peaks at 21.20 conforms the methyl group on pyranone ring. Compound (IVA-j) have shown $^{13}C$ NMR data at 168.20 conforms the formation of oxime, remaining have appeared similar to the compounds for (IIa-j).

Final conformation of derivative have been done by DIPMS data, electron impact mass spectra showed an accurate molecular ion peak at m/z 281.3256, 297.3276, 297.3276, 314.0127, 314.0127, 326.0350, 326.0350, 325.0420 for title compounds IIa-j, respectively, and electron impact mass spectra showed an accurate molecular ion peak at m/z 296.060, 312.055, 312.055, 312.055, 320.022, 330.022, 330.022, 341.046, 341.046, and 340.052 for title compounds IVA-j, respectively.

The synthesized compounds were evaluated for in vitro antibacterial and antifungal activity against various strains Gr(+) bacterial strains: B. subtilis; S. aureus Gr(-) bacterial strains: E. coli; P. aeruginosa, fungal strains: S. cerevisiae, Ca. albicans, An: A. niger. The concentration of test compounds were 100 μg/mL. Solvent used DMF. NA = Not active.

### Table 1. Antibacterial and antifungal activity of synthesized novel series of 4-methyl-2-oxo-pyranyl-phenothiazines (IIa-j) derivatives by cup-plate (agar cup) method*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Bacterial strains</th>
<th>Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gr (+)</td>
<td>Gr (-)</td>
</tr>
<tr>
<td>IIa</td>
<td>-H</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>IIb</td>
<td>-2OH</td>
<td>NA</td>
<td>10</td>
</tr>
<tr>
<td>IIc</td>
<td>-3OH</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td>IID</td>
<td>-4OH</td>
<td>12</td>
<td>NA</td>
</tr>
<tr>
<td>IIe</td>
<td>-2Cl</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>IIff</td>
<td>-3Cl</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>IIg</td>
<td>-4Cl</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>IIf</td>
<td>-3NO$_2$</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>IIh</td>
<td>-4NO$_2$</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>IIII</td>
<td>-4COOH</td>
<td>NA</td>
<td>9</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Gresiofluvin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Gr (+) bacterial strains: Bs: B. subtilis, Sa: S. aureus Gr (-) bacterial strains: Ec: E. coli, Pa: P. aeruginosa, Fungal strains: Sc: S. cerevisiae, Ca: C. albicans, An: A. niger. The concentration of test compounds were 100 μg/mL. Solvent used DMF. NA = Not active.

### Table 2. Antibacterial and antifungal activity of synthesized novel series of 4-methylpyrans (2,3-β) phenothiazine-2(1H)-one oxime (IVA-j) derivatives by cup-plate (agar cup) method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Bacterial strains</th>
<th>Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gr (+)</td>
<td>Gr (-)</td>
</tr>
<tr>
<td>IVA</td>
<td>-H</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>IVB</td>
<td>-2OH</td>
<td>NA</td>
<td>10</td>
</tr>
<tr>
<td>IVc</td>
<td>-3OH</td>
<td>9</td>
<td>NA</td>
</tr>
<tr>
<td>IVd</td>
<td>-4OH</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>IVe</td>
<td>-2Cl</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>IVf</td>
<td>-3Cl</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>IVg</td>
<td>-4Cl</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>IVh</td>
<td>-3NO$_2$</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>IVi</td>
<td>-4NO$_2$</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>IVj</td>
<td>-4COOH</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Gresiofluvin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Gr (+) bacterial strains: Bs: B. subtilis, Sa: S. aureus Gr (-) bacterial strains: Ec: E. coli, Pa: P. aeruginosa, Fungal strains: Sc: S. cerevisiae, Ca: C. albicans, An: A. niger. The concentration of test compounds were 100 μg/mL. Solvent used DMF. NA = Not active.
COOH) groups. The results showed that compounds (IIIa), (IIIe), (IIib)(IIIj), (IVa), (IVf) and (IVi) exhibited comparable antibacterial and antifungal activity with the standard antibiotics ciprofloxin and greselleitin. It has been observed that compound (IVa) show better antibacterial and antifungal activity compare to individual coumarin and phenothiazine and compounds (IIIa) and (IVA) (contains no substitution at ring 4) were shown better activity compare to rest of compounds with various substitution like -OH, -Cl, -NO2 and -COOH at different positions like ortho, meta, and para.

4. Conclusions

In this paper, we report the synthesis and antimicrobial activity of novel series of 4-methyl-2-oxo-pyranyl-phenothiazine (IIIa-j) and 4-methylpyrano-(2,3-j)-phenothiazine-2(11H)-one oxime (IVA-j). The preliminary in vitro antimicrobial activity of these novel series of derivative has evidenced that some of newly synthesized derivatives have shown very prominent potential as antimicrobial agents. The possible improvement of antimicrobial activity of these derivatives can be further modified based on modulation of ring substituent and/or additional fictionalization warrants further investigation. In summary, we have identified novel ring substituent and/or additional fictionalization warrants derivatives can be further modified based on modulation of ring substituent and/or additional fictionalization warrants further investigation. In summary, we have identified novel ring substituent and/or additional fictionalization warrants.

Acknowledgement

Authors are thankful to Dr. Vipin Bhari Gupta, director Bhanwar Nahata College of Pharmacy, Mandsaur (Madhya Pradesh) for providing necessary facilities.

References