Synthesis and biological activity of 2-oxo-azetidine derivatives of phenothiazine

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(Received May 7, 2011; Revised June 15, 2011; Accepted June 20, 2011)

Abstract: We have synthesized of N-[2-(10H-phenothiazinyl)ethyl]-4-(phenyl)-3-chloro-2-oxo-1-iminoazetidine 4(a-m). The structures of all the newly synthesized compounds were confirmed by IR, 1H NMR, 13C NMR and FAB-Mass and chemical methods. All synthesized compounds were evaluated for their antibacterial, antifungal and antitubercular activity which displayed acceptable results.

Keywords: Synthesis; biological activity; 2-azetidinone, phenothiazine.

1. Introduction

Phenothiazines are amongst the most frequently encountered heterocycles in compounds of biological interest. They have been shown to possess a broad spectrum of biological activity depending on their particular structure. Insect, its constitute the largest group of psychoactive clinically used compounds, phenothiazine derivatives possess several other biological activities including antibacterial, antifungal, antiproliferative, antipsychotic, anti-inflammatory and antiparkinsonian activities. Tuberculosis is the leading infectious disease among adults and youth, one third of the world population infected with mycobacterium tuberculosis. Recent studies have shown the synthesis of some new phenothiazine candidates as antitubercular agents. 2-Azetidinone skeleton is well established as the pharmacophore of β-lactam antibiotics. β-lactam antibiotics are the most widely employed class of antibiotics. The important and structural diversity of biologically active β-lactam antibiotics led to the development of many novel methods for the construction of appropriately substituted azetidine with attendant control of functional group and stereochemistry. Azetidine derivatives are reported to show a variety of antimicrobial, antitubercular, anticonvulsant, antiinflammatory and cardiovascular activities. These all activities showed that the minor change in the substitution pattern activities of azetidine derivatives have enhanced dramatically so our research group decide to synthesized a new series azetidine derivatives with several substitutions.

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2. Results and discussion

We have synthesized of \(N\)-[2-(10\(H\)-phenothiazinyl)ethyl]4-(phenyl)-3-chloro-2-oxo-1-iminoazetidine compounds 4(a-m) as shown in scheme 1. Phenothiazine on reaction with Cl(CH\(_2\))\(_2\)Br at room temperature gave 1-(2-chloroethyl)-10\(H\)-phenothiazine, compound 1. The compound 1 on the reaction with hydrazine hydrate at room temperature, yielded \(N\)-[2-(10\(H\)-phenothiazinyl)ethyl]-hydrazine, compound 2. The compound 2 on further reaction with several substituted aromatic aldehydes produced \(N\)-[2-(10\(H\)-phenothiazinyl)ethyl]-\(N\)'-[phenyl]methylene]-hydrazine, compounds 3(a-m). The compounds 3(a-m) on treatment with ClCH\(_2\)COCl in the presence of Et\(_3\)N furnished final products compounds 4(a-m). The structures of all the newly synthesized compounds were confirmed by IR, \(^1\)H NMR, \(^{13}\)C NMR and FAB-Mass and chemical methods. All synthesized compounds were evaluated for their antibacterial, antifungal and antitubercular activity which displayed acceptable activity in Table 1 and 2.

![Scheme 1: Synthesis of compounds 1, 2, 3(a-m) and 4(a-m).](image)

**2.1. Spectral data**

**2.1.1. Infrared spectral analysis**

The appearance of an absorption band in the IR spectrum of the compound 1 for (N-CH\(_2\)) and (C-Cl) at 1262 and 771 cm\(^{-1}\) respectively have been found and NH proton of phenothiazine has been disappeared (at 3462 cm\(^{-1}\)). The appearance of absorption band in IR spectrum of compound 2 were appeared for (NH) and (NH\(_2\)) at 3348 and 3430 cm\(^{-1}\) respectively. Appearance of absorption band in the spectra of compounds 3(a-m) for (N=C) was found in range of 1542.-1585 cm\(^{-1}\) which was a strong evidence for the synthesis of compounds 3(a-m). In the IR spectra of compounds 4(a-m), appearances of absorption peak for (CO cyclic) was found in range of 1732-1753 cm\(^{-1}\) which was suggesting the cyclization. This fact also supported by the disappearance of the peak of (N=CH) in the compounds 3(a-m).

**2.1.2. \(^1\)H NMR spectral analysis**

In the \(^1\)H NMR spectrum of the compound 1, a new signal appeared for N-CH\(_2\) at (\(\delta\)) 4.77 ppm. In the \(^1\)H NMR spectrum of the compound 2 showed two signals for NH and NH\(_2\) at (\(\delta\)) 7.61 and 5.69 ppm respectively. Presence of signals of NH and NH\(_2\) confirm the synthesis of compound 2. In the
compounds 3(a-m), the $^1$H NMR spectra showed a singlet for N=CH in the range of (δ) 7.68-8.08 ppm which provide a strong evidence for presence of benzylidine type proton and also supported by disappearance of NH$_2$ proton in the 1H NMR spectrum of compound 2. In the $^1$H NMR spectra of compounds 4(a-m) showed a strong signals for (CH-Cl) and (N-CH) of azetidine ring in the range of (δ) 4.20-4.49 and 4.68-4.98 ppm respectively and fact also supported by disappearance of N=CH signal in the compounds 3(a-m).

### 2.1.3. $^{13}$C NMR spectral analysis

$^{13}$C NMR spectra of the compound 1 showed a strong signal of N-CH$_2$ at (δ) 54.8 ppm. The signal for N=CH appeared in the range of (δ) 152.6-158.8 ppm in the $^{13}$C NMR spectra of compounds 3(a-m) which confirm the presence of carbon which is doubly bonded to nitrogen. In the spectra of compounds 4(a-m) three new characteristic signals were found for (CH-Cl), (N-CH) and cyclic CO in the range of (δ) 47.6-58.6, 57.7-67.7 and 169.5-177.6 ppm respectively. It is strong evidence of the cyclization and also supported by disappearance of N=CH signal of $^{13}$C NMR spectra of the compound 3(a-m).

### 2.1.4. FAB mass spectral analysis

FAB-Mass spectra of compounds 1, 2, 3(a-m) and 4(a-m) were showed appropriate parent ion peaks corresponding to their molecular formula weight respectively.

### 2.2 Biological activities

Compounds 4(a-m) were prepared and screened for their antimicrobial and antitubercular activities data (as shown in Table 1 and 2) revealed that all the synthesized compounds 4(a-m) have a structure activity relationship (SAR) because activity of compounds varies with substitution. Nitro group containing compounds (4h, 4i and 4j) showed higher activity than chloro (4c, 4d), or bromo group containing compounds (4e, 4f). Chloro and bromo derivatives also have higher activity than other rested compounds. On the basis of SAR, concluded that the activity of compounds depends on electron withdrawing nature of the substituted groups. The sequence of the activity is following

NO$_2$ > Cl > Br > OH > OCH$_3$ > CH$_3$

### Table 1. Antifungal and antibacterial activities of compounds 4(a-m) with MIC value (µg/mL).

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<tr>
<th>Comp.</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
<th>A. niger</th>
<th>A. flavus</th>
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<td>6.25</td>
<td>3.25</td>
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<td>12.5</td>
<td>6.25</td>
<td>9.25</td>
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*strept.= streptomycin standard for all bacteria strain and griso. = griseofulvin standard for all fungi strain.
Synthesis and biological activity of 2-oxo-azetidine derivatives of phenothiazine

The investigation of antimicrobial (antibacterial, antifungal and antitubercular) data revealed that the compounds (4c), (4d), (4e), (4f), (4h), (4i) and (4j) displayed high activity in the series, the compounds (4b), (4g) and (4m) showed moderate activity and rest compounds showed less activity against all the strains compared with standard drugs.

**Table 2.** Antitubercular activity of compounds 4(a-m) with MIC value (µg/mL).

<table>
<thead>
<tr>
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<td>4i</td>
<td>2.50</td>
<td>4l</td>
<td>&gt;12.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Comp. = compound; Conc. = concentration (µg/ml). Isoniazid and rifampicin were used as standards, MIC values 1.25, 2.50 µg/ml respectively for M. tuberculosis.

3. Conclusion

Compounds 4(a-m) were synthesized by an efficient route and screened for their antibacterial, antifungal and antitubercular activity against selected microorganisms. The investigation of antimicrobial data revealed that the compounds (4c), (4d), (4e), (4f), (4h), (4i) and (4j) displayed highly active in the series, compounds (4b), (4g) and (4m) showed moderate activity and rest compounds showed less activity against all the strains compared with standard drugs.

4. Experimental

Melting points were taken in open capillaries and are uncorrected. Progress of reaction was monitored by silica gel-G coated TLC plates using MeOH:CHCl\(_3\) system (1:9). The spot was visualized by exposing dry plate at iodine vapours chamber. IR spectra were recorded in KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (\(\nu_{\text{max}}\) in cm\(^{-1}\)) and \(^1\)H NMR and \(^{13}\)C NMR spectra were measured on a Brucker DRX-300 spectrometer in CDCl\(_3\) at 300 and 75 MHz respectively using TMS as an internal standard. All chemical shifts were reported on \(\delta\) scale. The FAB mass spectra were recorded on a Jeol SX–102 mass spectrometer. Elemental analyses were performed on a Carlo Erba–1108 analyzer. The analytical data of all the compounds were highly satisfactory. For column chromatographic purification of the products, Merck silica Gel 60 (230-400 Mesh) was used. The reagent grade chemicals were purchased from the commercial sources and further purified before use.

4.1. Procedure for the synthesis of compound 1.

A mixture of phenothiazine and 1-bromo-2-chloroethane (1:1 mole) was dissolved in acetone at room temperature. The reaction mixture was continuously stirred on a magnetic stirrer for about 8 h. The product was filtered and purified over a column chromatography using solvent system acetone : chloroform (8 : 2) as eluent. The purified product was recrystallized from ethanol at room temperature to yield compound 1.

**1-(2-chloroethyl)-10H-phenothiazine (1).** Yield: 60%; mp 160-165 °C; Anal. Calcd for C\(_{14}\)H\(_{12}\)NSCl: C,64.23, H,4.62, N,5.35%; found C,64.20, H,4.53, N,5.31%; IR (cm\(^{-1}\)): 3034, 2936 (CH), 1552 (C=C), 1262 (N-CH\(_2\)), 681 (C-S-C), \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\): 3.48 (t, 2H, \(J=7.42\) Hz, CH\(_2\)-Cl), 3.77 (t, 2H, \(J=7.42\) Hz, N-CH\(_2\)), 6.91-8.21 (m, 8H, Ar-H); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz) \(\delta\): 54.8 (N-CH\(_2\)), 46.3 (CH\(_2\)-Cl), 117.7, 121.7, 126.5, 127.7, 146.7, 148.8 (Ar); Mass (FAB): 262M\(^+\).
4.2. Procedure for the synthesis of compound 2.

A mixture of compound 1 and hydrazine hydrate (1:1 mole) was dissolved in acetone at room temperature. The reaction mixture was continuously stirred on a magnetic stirrer for about 2-4 h then kept on a steam bath for about 2.3-5 h. The products were filtered and cooled at room temperature. The filtered products were purified over a column chromatography acetone : chloroform (7 : 7) as eluent and recrystallized from ethanol at room temperature to yield compounds 3(a-m).

N-[2-(10H-phenothiazinyl)ethyl]-N’-[phenyl)methylidene]-hydrazine (3a). Yield: 66%; mp 150-153 °C; Anal. Calcd for C_{25}H_{22}N_{4}S: C,73.01, H,5.54, N,12.16%; found C,72.95, H,5.50, N,12.11%; IR: 1545 (N=CH), 3362 (NH); 1H NMR (CDCl₃, 300 MHz) δ: 3.45 (m, 2H, CH₂-NH), 5.82 (N-CH), 3.95 (t, 2H, J = 7.51 Hz, N-CH₃), 7.78 (s, 1H, NH), 7.86 (s, 1H, N=CH), 6.49-7.92 (m, 12H, Ar-H); 13C NMR (CDCl₃, 75 MHz) δ: 45.2 (CH₂-NH), 56.3 (N-CH₃), 158.8 (N=CH), 116.9, 120.8, 122.9, 123.7, 124.8, 126.9, 127.7, 127.8, 128.6, 135.8, 145.4, 146.8, 147.7 (Ar); Mass (FAB): 380M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N’-[4-chlorophenyl)methylidene]-hydrazine (3b). Yield: 67%; mp 156-162 °C; Anal. Calcd for C_{25}H_{21}ClN_{4}S: C,66.39, H,4.77, N,11.02%; found C,66.32, H,4.74, N,11.02%; IR: 1545 (N=CH), 3364 (NH); 1H NMR (CDCl₃, 300 MHz) δ: 3.47 (m, 2H, CH₂-NH), 5.80 (N-CH), 3.95 (t, 2H, J = 7.64 Hz, N-CH₃), 8.01 (s, 1H, NH), 8.08 (s, 1H, N=CH), 6.44-7.92 (m, 12H, Ar-H); 13C NMR (CDCl₃, 75 MHz) δ: 45.7 (CH₂-NH), 56.3 (N-CH₃), 157.6 (N=CH), 115.7, 121.2, 124.6, 126.9, 127.7, 127.8, 128.6, 135.8, 145.8, 147.7 (Ar); Mass (FAB): 380M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N’-[3-chlorophenyl)methylidene]-hydrazine (3c). Yield: 65%; mp 158-161 °C; Anal. Calcd for C_{25}H_{21}ClN_{4}S: C,66.39, H,4.77, N,11.06%; found C,66.37, H,4.71, N,11.00%; IR: 744 (C-Cl), 1584 (N=CH), 3374 (NH); 1H NMR (CDCl₃, 300 MHz) δ: 3.52 (m, 2H, CH₂-NH), 4.01 (t, 2H, J = 7.65 Hz, N-CH₃), 8.06 (s, 1H, NH), 8.11 (s, 1H, N=CH), 6.41-7.88 (m, 12H, Ar-H); 13C NMR (CDCl₃, 75 MHz) δ: 45.2 (CH₂-NH), 56.3 (N-CH₃), 157.6 (N=CH), 116.8, 122.9, 123.7, 124.8, 125.7, 126.8, 128.9, 130.7, 135.5, 146.4, 149.4 (Ar); Mass (FAB): 380M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N’-[4-bromophenyl)methylidene]-hydrazine (3d). Yield: 74%; mp 158-161 °C; Anal. Calcd for C_{25}H_{21}BrN_{4}S: C,59.43, H,4.27, N,9.90%; found C,59.38, H,4.21, N,9.87%; IR: 734 (C-Br), 1571 (N=CH), 3378 (NH); 1H NMR (CDCl₃, 300 MHz) δ: 3.47 (m, 2H, CH₂-NH), 3.92 (t, 2H, J = 7.61 Hz, N-CH₃), 7.97 (s, 1H, NH), 8.09 (s, 1H, N=CH), 6.39-7.96 (m, 12H, Ar-H); 13C NMR (CDCl₃, 75 MHz) δ: 47.6 (CH₂-NH), 58.2 (N-CH₃), 158.8 (N=CH), 116.9, 120.8, 122.9, 126.8, 127.8, 128.8, 132.4, 138.7, 148.4, 150.9 (Ar); Mass (FAB): 424M⁺.
N-[2-(10H-phenothiazinyl)ethyl]-N'-[(3-bromophenyl)methylidene]-hydrazine (3f). Yield: 64%; mp 151-154 °C; Anal. Calcd for C_{32}H_{28}BrN_{4}S: C,64.59, H,4.64, N,14.34%; found C,64.55, H,4.62, N,14.32%; IR: 848 (C-N), 1534 (NH), 3358 (NH); ^1H NMR (CDCl_3, 300 MHz): δ: 3.38 (m, 2H, CH_2-NH), 3.96 (t, 2H, J = 7.64 Hz, N-CH_3), 8.02 (s, 1H, NH), 8.17 (s, 1H, N=CH), 6.39-7.96 (m, 12H, Ar-H); ^13C NMR (CDCl_3, 75 MHz): δ: 46.5 (CH_3-NH), 56.8 (N=CH_2), 155.8 (N=CH), 151.9, 151.0, 150.1, 149.4, 149.2 (Ar); Mass (FAB): 390M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N'-[(3-nitrophenyl)methylidene]-hydrazine (3i). Yield: 63%; mp 162-166 °C; Anal. Calcd for C_{32}H_{28}N_4O_4S: C,64.59, H,4.64, N,14.34%; found C,64.55, H,4.62, N,14.32%; IR: 848 (C-N), 1534 (NH), 3358 (NH); ^1H NMR (CDCl_3, 300 MHz): δ: 3.35 (m, 2H, CH_2-NH), 3.85 (t, 2H, J = 7.56 Hz, N-CH_3), 7.79 (s, 1H, NH), 7.78 (s, 1H, N=CH), 6.54-7.85 (m, 12H, Ar-H); ^13C NMR (CDCl_3, 75 MHz): δ: 45.9 (CH_3-NH), 54.2 (N=CH_2), 55.9 (OCH_3), 154.6 (N=CH), 118.4, 121.5, 125.7, 126.4, 130.8, 135.1, 138.6, 142.2, 149.3, 158.9 (Ar); Mass (FAB): 390M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N'-[(4-methoxyphenyl)methylidene]-hydrazine (3k). Yield: 61%; mp 147-150 °C; Anal. Calcd for C_{32}H_{28}N_4O_5S: C,70.37, H,5.63, N,11.19%; found C,70.32, H,5.60, N,11.12%; IR: 1559 (N=CH), 2950 (OCH_3), 3358 (NH); ^1H NMR (CDCl_3, 300 MHz): δ: 4.21 (s, 3H, OCH_3), 3.31 (m, 2H, CH_2-NH), 3.85 (t, 2H, J = 7.56 Hz, N-CH_3), 7.79 (s, 1H, NH), 7.78 (s, 1H, N=CH), 6.54-7.85 (m, 12H, Ar-H); ^13C NMR (CDCl_3, 75 MHz): δ: 45.9 (CH_3-NH), 54.2 (N=CH_2), 55.9 (OCH_3), 154.6 (N=CH), 118.4, 121.5, 125.7, 126.4, 130.8, 135.1, 138.6, 142.2, 149.3, 158.9 (Ar); Mass (FAB): 375M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N'-[(4-methylphenyl)methylidene]-hydrazine (3l). Yield: 60%; mp 142-146 °C; Anal. Calcd for C_{32}H_{28}N_4O_4S: C,73.50, H,5.88, N,11.58%; found C,73.42, H,5.81, N,11.52%; IR: 1542 (N=CH), 2924 (CH_3), 3348 (NH); ^1H NMR (CDCl_3, 300 MHz): δ: 2.39 (s, 3H, CH_3), 3.19 (m, 2H, CH_2-NH), 3.71 (t, 2H, J = 7.51 Hz, N-CH_3), 7.84 (s, 1H, NH), 7.98 (s, 1H, N=CH), 6.52-8.03 (m, 12H, Ar-H); ^13C NMR (CDCl_3, 75 MHz): δ: 27.6 (CH_3-NH), 46.3 (CH_2-NH), 54.6 (N=CH_2), 152.6 (N=CH), 117.2, 123.5, 124.7, 126.2, 127.8, 129.6, 136.0, 138.1, 143.5, 149.8 (Ar); Mass (FAB): 359M⁺.

N-[2-(10H-phenothiazinyl)ethyl]-N'-[(4-hydroxyphenyl)methylidene]-hydrazine (3m). Yield: 64%; mp 140-143 °C; Anal. Calcd for C_{32}H_{28}O_3N_4S: C,69.77, H,5.29, N,11.62%; found C,69.72, H,5.21, N,11.60%; IR: 1557 (N=CH), 3362 (NH), 3464 (OH); ^1H NMR (CDCl_3, 300 MHz): δ: 3.38 (m, 2H, CH_2-NH), 3.94 (t, 2H, J = 7.46 Hz, N-CH_3), 4.24 (s, 1H, OH), 7.98 (s, 1H, NH), 8.05 (s, 1H, N=CH), 6.49-7.72 (m, 12H, Ar-H); ^13C NMR (CDCl_3, 75 MHz): δ: 47.5 (CH_3-NH), 58.2 (N=CH_2), 157.9 (N=CH), 115.7, 122.6, 124.9, 125.7, 127.8, 128.7, 137.3, 139.7, 145.8, 156.6 (Ar); Mass (FAB): 403M⁺.
4.4. General procedure for the synthesis of compounds 4(a-m).

A mixture of compounds 3(a-m) and chloroacetyl chloride in the presence of Et₃N (1:1 mole) was dissolved in acetonitrile at room temperature and allowed to react. The reaction mixture was first continuously stirred on a magnetic stirrer for about 2-3 h then kept on a steam bath for about 2.3-4.2 h. The products were filtered and cooled at room temperature. The filtered products were purified over a column chromatography: chloroform (7:3) as eluent and recrystallized from ethanol at room temperature to yield compounds 4(a-m).

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(phenyl)-3-chloro-2-oxo-1-iminoazetidine (4a). \]
Yield: 68%; mp 142-145 °C; Anal. Calcd for C₂₃H₂₃N₃SO: C, 71.47, H, 5.21, N, 10.47%; found C, 71.41, H, 5.18, N, 10.41%; IR: 1334 (C=N), 1732 (C=O cyclic), 2927 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.30 (m, 2H, CH₂-NH), 3.90 (t, 2H, J = 7.40 Hz, N-CH₂), 4.34 (d, 1H, J = 5.0 Hz, CH-Cl), 4.81 (d, 1H, J = 5.0 Hz, N-CH₂), 6.52-7.84 (m, 13H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 42.1 (CH₂-NH), 53.0 (N-CH₃), 47.6 (CH-Cl), 57.9 (N-CH), 171.5 (CO cyclic), 114.5, 118.9, 123.5, 126.7, 128.5, 129.1, 130.7, 136.2, 145.8, 149.7 (Ar); Mass (FAB): 386M⁺.

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(4-chlorophenyl)-3-chloro-2-oxo-1-iminoazetidine (4b). \]
Yield: 64%; mp 144-146 °C; Anal. Calcd for C₂₃H₂₂N₃SOCl₂: C, 60.52, H, 4.19, N, 9.20%; found C, 60.48, H, 4.12, N, 9.16%; IR: 769 (C=Cl), 1336 (C-N), 1748 (CO cyclic), 2920 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.32 (m, 2H, CH₂-NH), 3.98 (t, 2H, J = 7.45 Hz, N-CH₂), 4.41 (d, 1H, J = 5.10 Hz, CH-Cl), 4.98 (d, 1H, J = 5.10 Hz, N-CH₂), 6.51-8.12 (m, 12H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 41.3 (CH₂-NH), 55.2 (N-CH₃), 53.9 (CH-Cl), 65.6 (N-CH), 177.6 (CO cyclic), 117.5, 122.8, 124.9, 126.7, 127.7, 129.9, 130.8, 138.8, 146.3, 149.4 (Ar); Mass (FAB): 456M⁺.

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(3-chlorophenyl)-3-chloro-2-oxo-1-iminoazetidine (4c). \]
Yield: 64%; mp 140-142 °C; Anal. Calcd for C₂₃H₂₂N₃SOCl₂: C, 60.52, H, 4.19, N, 9.20%; found C, 60.44, H, 4.13, N, 9.14%; IR: 774 (C=Cl), 1342 (C-N), 1750 (CO cyclic), 2929 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.35 (m, 2H, CH₂-NH), 3.88 (t, 2H, J = 7.45 Hz, N-CH₂), 4.43 (d, 1H, J = 5.10 Hz, CH-Cl), 4.91 (d, 1H, J = 5.10 Hz, N-CH₂), 6.53-8.05 (m, 12H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 44.9 (CH₂-NH), 52.6 (N-CH₃), 55.7 (CH-Cl), 64.8 (N-CH), 177.5 (CO cyclic), 115.8, 124.2, 125.8, 127.7, 129.3, 130.9, 131.6, 135.1, 135.8, 141.6, 145.7, 148.8 (Ar); Mass (FAB): 456M⁺.

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(2-chlorophenyl)-3-chloro-2-oxo-1-iminoazetidine (4d). \]
Yield: 64%; mp 138-141 °C; Anal. Calcd for C₂₃H₂₂N₃SOCl₂: C, 60.52, H, 4.19, N, 9.20%; found C, 60.41, H, 4.11, N, 9.18%; IR: 774 (C=Cl), 1339 (C-N), 1742 (C=O cyclic), 2922 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.30 (m, 2H, CH₂-NH), 3.92 (t, 2H, J = 7.40 Hz, N-CH₂), 4.39 (d, 1H, J = 5.15 Hz, CH-Cl), 4.96 (d, 1H, J = 5.15 Hz, N-CH₂), 6.46-8.01 (m, 12H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 43.7 (CH₂-NH), 55.4 (N-CH₃), 57.8 (CH-Cl), 64.5 (N-CH), 177.6 (CO cyclic), 114.8, 119.0, 123.5, 125.8, 126.7, 128.7, 130.8, 136.7, 138.8, 145.8, 148.3, 150.4, (Ar); Mass (FAB): 456M⁺.

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(4-bromophenyl)-3-chloro-2-oxo-1-iminoazetidine (4e). \]
Yield: 61%; mp 151-154 °C; Anal. Calcd for C₂₃H₁₉N₃SOBrCl: C, 55.15, H, 3.82, N, 8.38%; found C, 55.11, H, 3.78, N, 8.34%; IR: 572 (C-Br), 1338 (C-N), 1742 (CO cyclic), 2892 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.31 (m, 2H, CH₂-NH), 3.91 (t, 2H, J = 7.46 Hz, N-CH₂), 4.39 (d, 1H, J = 5.05 Hz, CH-Cl), 4.88 (d, 1H, J = 5.05 Hz, N-CH₂), 6.48-7.89 (m, 12H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 40.9 (CH₂-NH), 53.4 (N-CH₃), 52.6 (CH-Cl), 62.3 (N-CH), 176 (CO cyclic), 115.8, 120.8, 124.3, 126.7, 127.3, 128.3, 133.2, 137.6, 141.3, 148.4 (Ar); Mass (FAB): 501M⁺.

\[ N-[2-(10H-phenothiazinyl)ethyl]-4-(3-bromophenyl)-3-chloro-2-oxo-1-iminoazetidine (4f). \]
Yield: 64; mp 153-155 °C; Anal. Calcd for C₂₃H₁₉N₃SOBrCl: C, 55.15, H, 3.82, N, 8.38%; found C, 55.10, H, 3.80, N, 8.31%; IR: 593 (C-Br), 1338 (C-N), 1746 (CO cyclic), 2890 (CH-Cl); \(^1\)H NMR (CDCl₃, 300 MHz) \( \delta \) 3.35 (m, 2H, CH₂-NH), 3.87 (t, 2H, J = 7.50 Hz, N-CH₂), 4.32 (d, 1H, J = 5.15 Hz, CH-Cl), 4.86 (d, 1H, J = 5.15 Hz, N-CH₂), 6.43-7.87 (m, 12H, Ar-H); \(^1^3^C\) NMR (CDCl₃, 75 MHz) \( \delta \) 46.1 (CH₂-
N-[2-(10H-phenothiazinyl)ethyl]-4-(4-methoxyphenyl)-3-chloro-2-oxo-1-iminoazetidine (4k).

Yield: 6%; mp 135-137 °C; Anal. Caled for C_{23}H_{19}N_{2}O_{5}Cl: C, 56.29; H, 3.92; N, 9.29%; found C, 56.31; H, 4.02; N, 9.31%; IR: 1500 (C-O), 1600 (C=O), 3100 (CH), 2920 (CH-CH); \( ^1 \text{H} \) NMR (CDCl\(_3\), 300 MHz): \( \delta \) 3.28 (s, 3H, CH\(_3\)-O), 7.64 (d, 2H, J = 7.45 Hz, N-CH\(_2\)), 4.20 (d, 1H, J = 4.90 Hz, CH-Cl), 4.87 (d, 1H, J = 5.0 Hz, N-CH), 6.26-7.92 (m, 12H, Ar-H); \( ^{13} \text{C} \) NMR (CDCl\(_3\), 75 MHz): \( \delta \) 41.9 (CH\(_2\)-NH), 50.2 (N-CH\(_2\)), 51.6 (CH-Cl), 55.6 (OCH\(_3\)), 65.4 (N-CH), 174.5 (CO, cyclic), 117.9, 122.4, 125.9, 128.7, 130.8, 136.5, 137.8, 139.8, 148.7, 155.9 (Ar); Mass (FAB): 452M\(^+\).

N-[2-(10H-phenothiazinyl)ethyl]-4-(4-hydroxyphenyl)-3-chloro-2-oxo-1-iminoazetidine (4n).

Yield: 60%; mp 132-135 °C; Anal. Caled for C_{23}H_{20}N_{2}O_{4}Cl: C, 63.07; H, 4.60; N, 9.59%; found C, 63.01; H, 4.53; N, 9.51%; IR: 1177 (C-O), 1357 (C-N), 1753 (CO cyclic), 2927 (CH-Cl), 3469 (OH); \( ^1 \text{H} \) NMR (CDCl\(_3\), 300 MHz): \( \delta \) 3.30 (m, 2H, CH\(_2\)-NH), 3.90 (t, 2H, J = 7.45 Hz, N-CH\(_2\)), 4.24 (s, 1H, OH), 4.49 (d, 1H, J = 4.85 Hz, CH-Cl), 4.98 (d, 1H, J = 4.85 Hz, N-CH), 7.09-8.1 (m, 12H, Ar-H); \( ^{13} \text{C} \) NMR (CDCl\(_3\), 75 MHz): \( \delta \) 43.3 (CH\(_2\)-NH), 52.7 (N-CH), 65.4 (N-CH), 174.5 (CO, cyclic), 117.9, 122.8, 124.8, 125.7, 127.7, 129.9, 135.9, 143.6, 149.8 (Ar); Mass (FAB): 436M\(^+\).
NMR (CDCl₃, 75 MHz) δ: 44.1 (CH₂-NH), 51.7 (N-CH₂), 56.9 (CH-Cl), 67.7 (N-CH), 176.5 (CO cyclic), 115.3, 124.4, 126.8, 129.7, 130.8, 135.8, 138.9, 146.4, 148.4, 158.3 (Ar); Mass (FAB): 438M⁺.

4.5. Biological study

The antibacterial, antifungal and antitubercular activities of compounds 4(a-m) has been assayed in vitro against selected Gram positive bacteria, Bacillus subtilis, Staphylococcus aureus and Gram negative bacteria, Escherichia coli, Klebsiella pneumoniae fungi, Aspergillus niger, Aspergillus flavus, Candida albicans Fusarium oxysporium and Mycobacterium tuberculosis H37Rv strain MIC values of compounds 4(a-m) were determined using filter paper disc diffusion method (antibacterial and antifungal activities) and L.J. medium (Conventional) method (antitubercular activity). Streptomycin and Griseofulvin used as standard for antibacterial and antifungal activity showed MIC range for all bacterial strain 1.25-6.25 µg/mL and for all fungal strain 6.25-12.5 µg/mL respectively and for antitubercular activity, Isoniazid and Rifampicin taken as standards. All standards also screened under the similar condition for comparison. Results of all given activities of above compounds were given in Table 1 and 2.

Acknowledgments

The authors are thankful to SAIF, Central Drugs Research Institute, Lucknow (India) for providing spectral and analytical data of the compounds. We are also thankful to Head, Department of Chemistry, Dr. H. S. Gour, University (A Central University), Sagar (India) for giving the facilities to carryout the work.

References

Synthesis and biological activity of 2-oxo-azetidine derivatives of phenothiazine


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