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N-substituted phenothiazine derivatives as potent antimicrobials: Synthesis and structural characterization

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ABSTRACT

*A series of N-substituted phenothiazine derived chloro compounds (**3a-3c**) and morpholine based compounds (**5a-5c**) were synthesized by base catalyzed reactions. The molecular structures of synthesized compounds were confirmed by using various spectral techniques viz., FT-IR, ^{1}H, ^{13}C NMR and mass spectroscopy. The strong stretching vibrational bands in the region 1696-1670 cm^{-1}in FT-IR spectral analysis confirms the presence of carbonyl moiety in the synthesized compounds. The presence of signals in the downfield region of proton NMR of **5a-5c** is due to methylene protons which confirms their formation. The antimicrobial investigations was carried out against two gram positive bacterial species such as Bacillus subtilis and Staphylococcusaureus. The antifungal activity was tested against AspergillusflavusandAspergillusniger. The biological results obtained suggests that the microbial growth inhibition was much higher in the chloro based N-phenothiazine derivatives (**3a-3c**) than its morpholine derivative (**5a-5c**).*

Keywords: Phenothiazine, Morpholine, Spectroscopy, Microbial growth inhibition.

INTRODUCTION

The phenothiazine has a three-ringed structure in which two benzene rings are connected by sulphur and a nitrogen atom (Figure 1) [1]. It is widely used as common building block for the synthesis of medicinally important compounds. *N*-substituted phenothiazine derivatives are potent biological heterocyclic compounds that possess wide range of biological applications such as anti-inflammatory, antihistaminic, antipsychotic, anticholinergic, antiemetic, sedative, tranquilizers, cytostatic, antimalarial, analgesic, antitubercular and antimicrobial agents [2, 3].

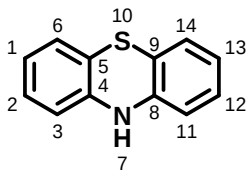


Figure 1: Structure of *10H*-phenothiazine back bone with atoms numbering

These activities represent the result of the interaction of phenothiazines with biological systems through their pharmacophoric substituents, the multicyclic ring system (π-π interaction, intercalation in DNA) or the lipophilic character allowing for the penetration through the biological membranes. The activities were examined by using various biological systems such as cell lines, viruses, bacteria, parasites, laboratory mice, rats and rabbits, and monolayer and bilayer membranes [4]. The *N*-alkylphenothiazines as ligands are used in metal complexes, but biological properties of these complexes are investigated in recent years.

Present work describes the synthesis of various *N*-substituted phenothiazine derivatives and characterization of synthesized compounds by FT-IR, NMR and MS techniques. The microbial growth inhibition activity of bacteria and fungi was carried out. The results indicated that compounds with halides showed better activity than other compounds (without halide).

MATERIALS AND METHODS

All the chemicals and solvents used were of AnalR grade procured from Merck and Loba chemical companies. Solvents were used as received. Infrared spectral measurements were performed using Perkin-Elmer Spectrometerversion 10.03.09.^{1}H and ^{13}C-NMR spectra of reported compounds were recorded on Agilent 400 MHz NMR spectrometer with TMS as internal standard. The compounds were dissolved in the deuterated solvent ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm). The mass spectra of the reported compounds was obtained by TOF-ES technique at 70 eV using ESI/APCI-hybrid mass spectrometer (Waters, USA).

Antimicrobial studies

Antibacterial activity

The antibacterial activity against two gram positive bacterial species namely, *Bacillus subtilis* and *Staphylococcus aureus*was performed by disc diffusion techniquemethod using nutrient agar medium [5].The test organisms were sub-cultured in the agar medium and were incubated for 24 hat 37 ^{o}C. Standard antibacterial drug, Ciprofloxacin was used for comparison. The discs of 4 mm diameter were soaked in the test solutions and then placed on medium previously seededwith organisms in Petri plates and incubated for 24 h at 37 ^{o}C. The inhibition zone circling eachdisc was measured and the results have been noted in the form of inhibition zones (n percentage.In order to clarify any effect ofsolvent (DMF) on the microbial screening, individual studies were performed with solution alone of DMF and they showed no activity against any microbial strains. The stock solution (1 mg/ml) of the test compounds was prepared in DMF [6]. The test was carried out intriplicate and the average is represented.

Antifungal Activity

The reported compounds were tested against two fungal species, *Aspergillus flavus*and *Aspergillus niger*. The test organisms were sub-cultured in potato dextrose agar medium and incubated at 37 ^{o}C for 48 h [7].Further procedure was similar as above. The standard antifungal drug, Griseofulvin was used for comparison.

General Synthesis

Synthesis of chloro phenothiazine ketone derivatives (3a-3c)

To a solution phenothiazine **(1)** in toluene (1 eq), chloroacetyl chloride derivatives **(2a-2c)** was added (2.4 eq) dropwise with stirring. The reaction mixture was refluxed at 60 ^{o}C for 3 hr. The completion of the reaction was monitored by time-to-time TLC. After the completion, the solvent was evaporated in rotary evaporator to obtain desired product. Recrystallization was done from ethanol.

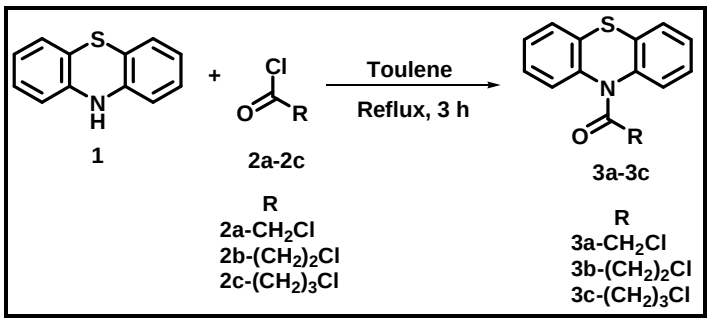
2-chloro-1-(10H-phenothiazin-10-yl)ethanone (3a)

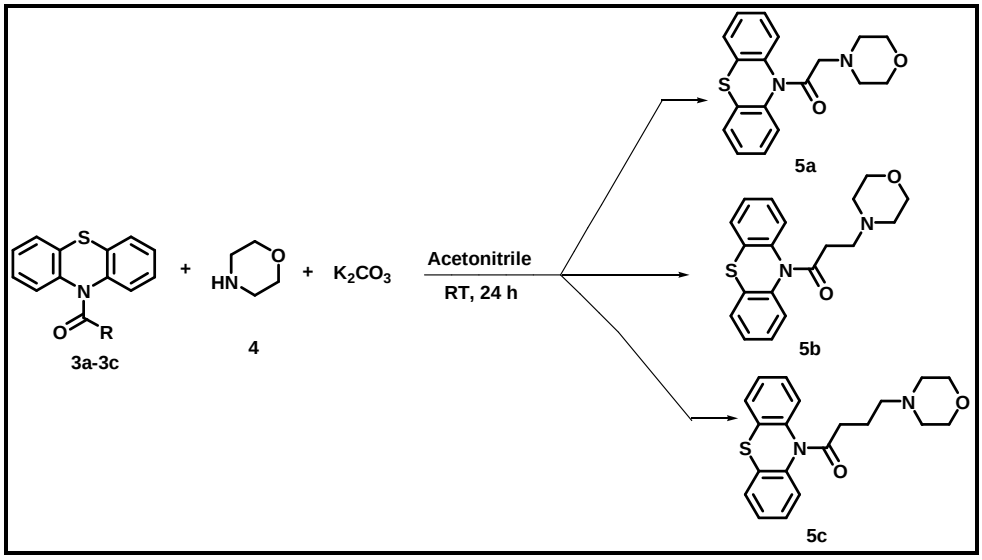
Yield: 73%; IR (KBr) cm^{-1}: 1696, 1673 (ss) (C=O), 3002 (ws) (Ar-H), 752.28 (s) (CH_2-Cl), 699.76 (ws) (Ar-S), 1340 (ss) (Ar-N); ^{1}H NMR ($CDCl_3$): δ 7.23-7.35 (m, 4H, Ar-H, J =7.99 Hz), δ 7.43-7.58 (m, 4H, Ar-H, J= 7.99Hz), δ 4.18 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2), 126.5, 127.3, 127.4, 128.1, 133.1, 137.9 (Ar-C), 165.4(C=O); MS: [M^+]=275.8976, [M^++2]=277.8938.

3-chloro-1-(10H-phenothiazin-10-yl)propan-1-one (3b)

Yield: 81%; IR (KBr) cm^{-1}: 1670 (s) (C=O), 2971 (b) (Ar-H), 766 (ws) (CH_2-Cl), 697(w)(Ar-S),1365(ss) (Ar-N); ^{1}H NMR($CDCl_3$): δ 7.21-7.34(m,4H,Ar-H, J=6.39 Hz), δ 7.43-7.51(m,4H,Ar-H, J=6.39 Hz),δ 3.78(t,2H,CH_2, J=6.79 Hz), δ 2.82(t,2H,$COCH_2$, J=6.79 Hz); ^{13}C NMR ($CDCl_3$): δ 39.5(CH_2),127.12,127.18,128.09, 138.16(Ar-C),169.0(C=O); MS: [M^+]=290.0453, [M^++2]=292.0453.

4-chloro-1-(10H-phenothiazin-10-yl)butan-1-one (3c)
Yield: 84%; IR(KBr) cm^{-1}: 1738 (w), 1675 (ss) (C=O), 2925 (ws) (Ar-H), 762 (ws) (CH_2-Cl), 690 (ss) (Ar-S), 1325 (ss) (Ar-N); ^1H NMR ($CDCl_3$): δ 7.21-7.34 (m, 4H, Ar-H, J=7.19 Hz), δ 7.43-7.51 (m, 4H, Ar-H, J= 7.59 Hz), δ 3.56 (t, 2H, COCH2, J=5.99 Hz), δ 2.62 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 44.3 (CH_2), 126.9, 127.0, 127.2, 128.0, 133.3, 138.6 (Ar-C), 171 (C=O); MS:[M^+]=303.9350, [M^++2]=305.9350.





Scheme 1: General synthetic route of 3a-3c and 5a-5c

Synthesis of morpholine based phenothiazine derivatives (5a-5c)
To a separate solution of **3a**, **3b** and **3c** (1 equivalent), 1.2 equivalent mass of morpholine separately was added along with 1.2 equivalent mass of potassium carbonate in acetonitrile. The above reaction mixture was stirred for 24 h at ambient temperature. The reaction towards completion was monitored by TLC. The solvent is evaporated under pressure in rotary evaporator. The product was extracted with ethyl acetate and this again evaporated to yield final product.

2-morpholino-1-(10H-phenothiazin-10-yl)ethanone (5a)
Yield: 69%; IR (KBr) cm^{-1}: 1671 (ss) (C=O), 2930 (w) (Ar-H), 729 (ws) (CH_2-Cl), 645 (ws) (Ar-S), 1352 (ws) (Ar-N); ^1H NMR ($CDCl_3$):δ 7.21-7.33 (m, 4H, Ar-H, J=6.39 Hz), δ 7.43-7.56 (m, 4H, Ar-H, J=6.39 Hz), δ 3.65 (t, 4H, OCH_2, J=4.79 Hz), δ 3.42 (s, 2H, $COCH_2$, J=5.99 Hz) δ 2.60 (t, 4H, N-CH_2, J=5.99 Hz); ^{13}C NMR ($CDCl_3$): δ 41.7 (CH_2), 126.5, 126.7, 127.0, 127.0, 127.2, 127.3, 127.9, 128.0 (Ar-C), 167.3 (C=O); MS: [M^+]=326.99, [M^++2]=328.99.

3-morpholino-1-(10H-phenothiazin-10-yl)propan-1-one (5b)
Yield: 71%; IR (KBr) cm^{-1}: 1601 (ss) (C=O), 3000 (w) (Ar-H), 800 (ws) (CH_2-Cl), 687 (ws) (Ar-S), 1380 (ws) (Ar-N); ^1H NMR ($CDCl_3$): δ 7.09-7.24 (m, 4H, Ar-H, J=6.39 Hz), δ 7.30-7.39 (m, 4H, Ar-H, J=6.39 Hz), δ 3.46 (t, 4H,

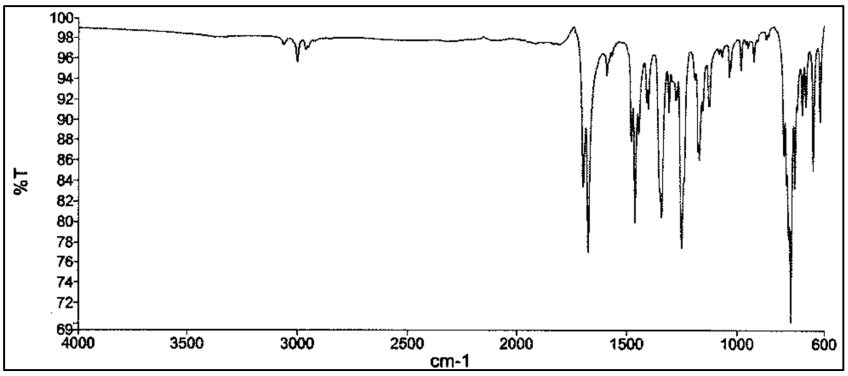
OCH_2, J=5.19 Hz), δ 2.70 (t, 4H, OCH_2, J=4.79 Hz), δ 2.15 (t, 4H, N-CH_2, J=4.79 Hz); ^{13}C NMR ($CDCl_3$): δ 42.3 (CH_2), 127.2, 127.6, 127.9, 128.1, 128.4, 128. 6, 128.9, 129.3 (Ar-C), 170.1 (C=O); MS: [M^+]=355.1078, [M^++2]=328.99.

4-morpholino-1-(10H-phenothiazin-10-yl)butan-1-one (5c)
Yield: 74%; IR (KBr) cm^{-1}:1736 (ss) (C=O), 2987 (w) (Ar-H), 762 (w) (CH_2-Cl), 610 (w) (Ar-S), 1374 (w) (Ar-N); ^{1}H NMR ($CDCl_3$): δ 7.17-7.31 (m, 4H, Ar-H), δ 7.40-7.49 (m, 4H, Ar-H), δ 3.60 (t, 4H, $COCH_2$, J=4.79 Hz), δ 2.65 (t, 4H, OCH_2, J=9.59 Hz), δ 2.30 (t, 4H, N-CH_2, J=4.39 Hz); ^{13}C NMR ($CDCl_3$): δ31.6(CH_2), 126.8,126.9, 127.2,127.8,128.0,133.2,138.5(Ar-C),170.7(C=O); MS: [M^+]=341.0949, [M^++2]=341.0949.

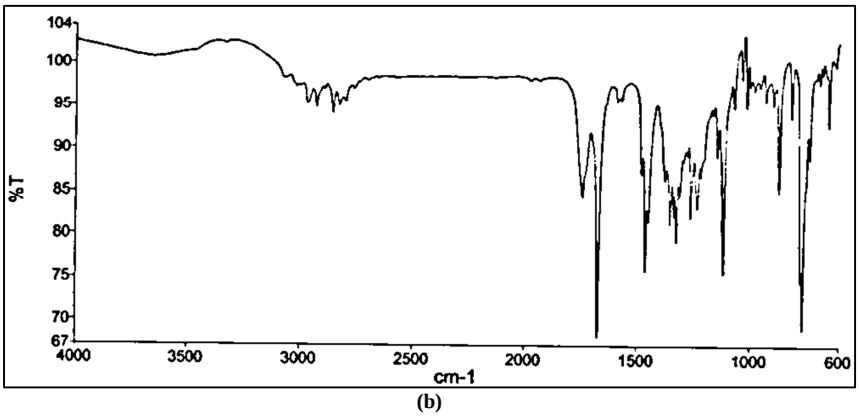
RESULTS AND DISCUSSION

The synthetic route of the *N*-Phenothiazine derivatives (**3a-3c** and **5a-5c**) are shown in Schemes 1 and 2. The molecular structures of the reported compounds were confirmed by IR, NMR and mass spectroscopic techniques.

Infrared Spectroscopy
The important stretching frequencies of the compounds were given in the Table 1. The representative spectra of compounds **3a** and **5a** are shown in figure 2 (a) and (b), respectively. The characteristic strong band of carbonyl group vibrations were observed in the region 1696-1670 cm^{-1} due to stretching modes of C=O moiety. The benzene ring owns six ring stretching modes of which the four with highest wavenumbers occurring near1600, 1580, 1490 and 1440 cm^{-1} are group vibrations [8].With substituents, the bands tend to shift to lower wavenumbers and the greater the number of substituents on the ring, broader the absorption regions [9].The sulfur bridged between the two aromatic rings experiences stretching mode of vibrations in the region 687-699 cm^{-1}[10].



(a)



(b)

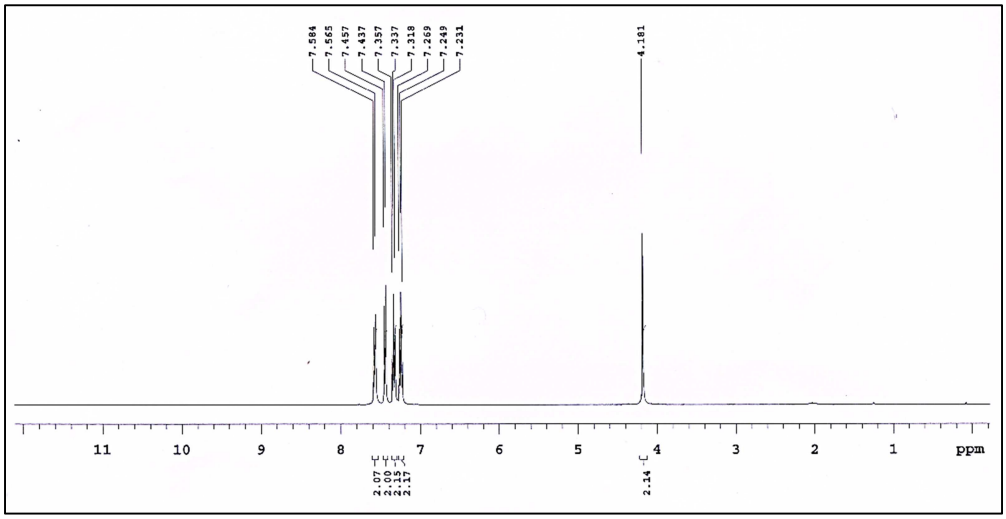
Figure 2: IR spectra of compounds (a) 3a and (b) 5a

Table 1: Important IR stretching frequencies of the synthesized compounds

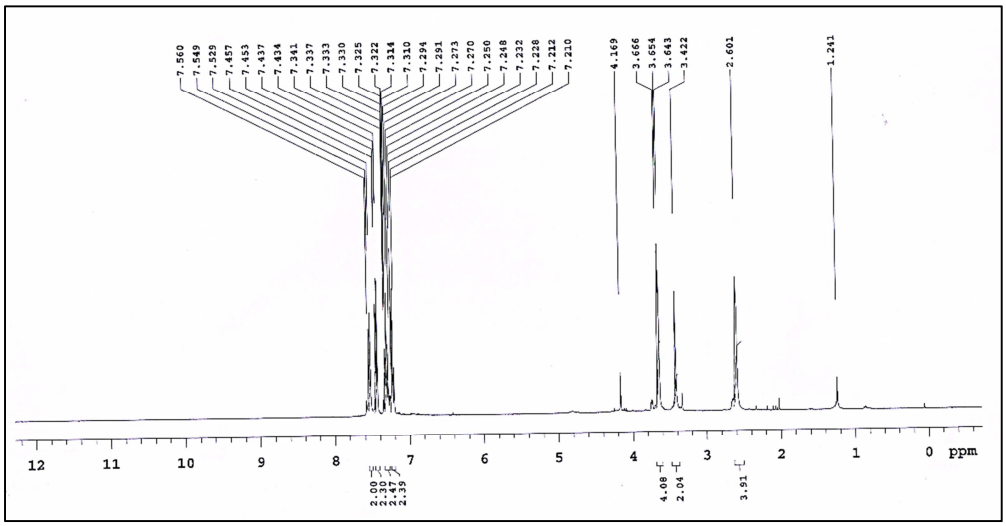
Compound	γ (C=O) (cm^{-1})	γ (Ar-S) (cm^{-1})	γ (Ar-N) (cm^{-1})	γ (CH_2-Cl) (cm^{-1})
3a	1696	699	1340	752
3b	1675	690	1325	762
3c	1670	697	1365	766
5a	1671	692	1352	729
5b	1683	683	1369	736
5c	1686	687	1374	762

NMR spectral analysis

The molecular structures of synthesized *N*-phenothiazine derivatives were further confirmed by NMR spectral analysis. The compound **3a** as a representative proton NMR spectrum of chloro series is shown in figure 3a.A singlet at δ = 4.18 ppm is due to the resonance of methylene protons. Similarly, the presence of signals in the downfield region of proton NMR of **5a-5c** is due to methylene protons which are deshielded by nitrogen and/or carbonyl group at either ends. This clearly indicates their formation and**5a** as a representative spectrum of morpholine series is shown in figure 3b.^{13}C NMR spectral analysis also supported for structural assignments of reported compounds.



(a)

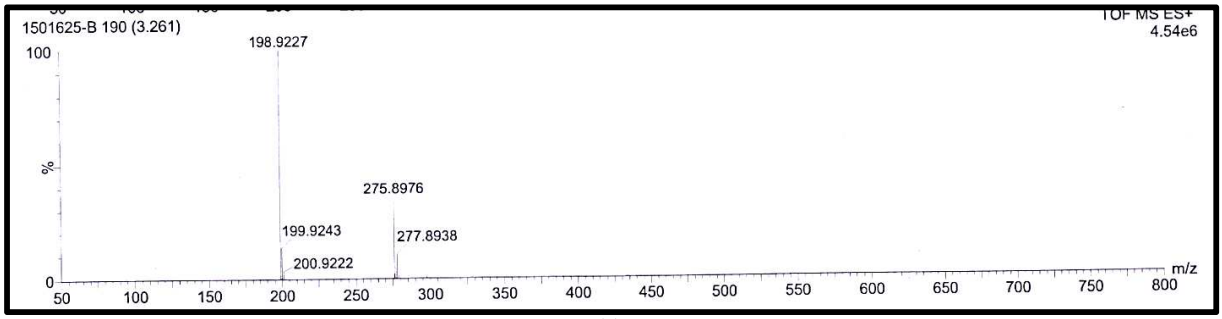


(b)

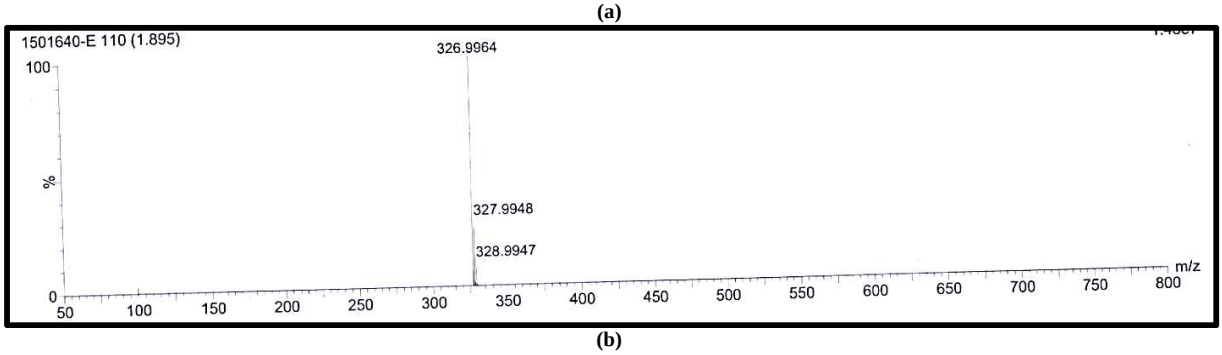
Figure 3:^{1}H NMR spectra of (a) 3a and (b) 5a

Mass Spectroscopy

The time-of-flight electron-spray mass spectral studies of the reported compounds were obtained on Agilent mass spectrometer. In the figure 4a and 4b, mass spectra of compounds **3a** and **5a** are showed as representative mass spectra for chloro and morpholine series, respectively. The molecular ion peaks of **3a** and **5a** are observed at *m/z* 275.89 and 326.99, respectively. In addition, a peak at *m/z* 277.89 is observed in **3a** due to chloro isotope (^{37}Cl).



(a)



(b)

Figure 4: Mass spectra of (a) 3a and (b) 5a

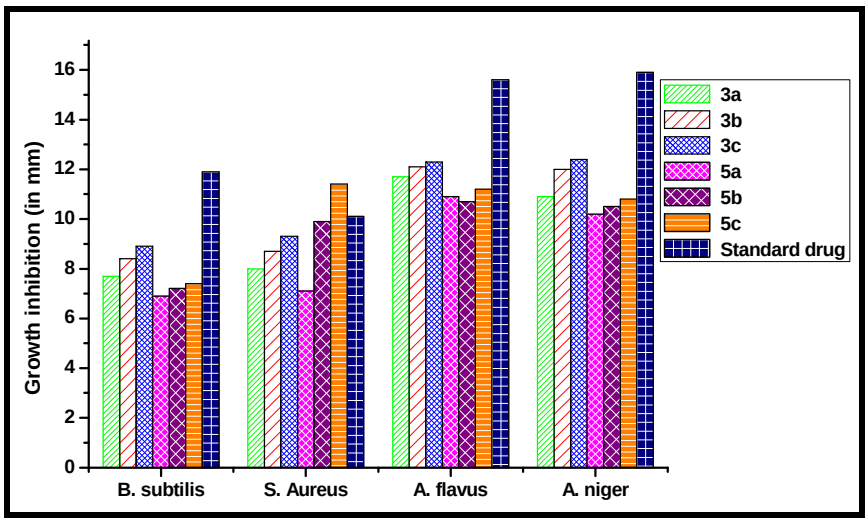


Figure 5: Graphical representation of microbial growth inhibition

Antimicrobial investigations

The synthesized compounds showed interesting antimicrobial activities against the selected species of bacteria and fungi. The graphical representation of microbial growth inhibition is shown in figure 5. The most promising antimicrobial activity of **3a-3c** was observed against *Staphylococcus aureus*, *Aspergillus flavus*and *Aspergillus niger*. Similarly, compounds **5a-5c** are moderately potent against all the tested microbes. The activities were compared with standard antibacterial and antifungal drugs such as Ciprofloxacin and Griseofulvin, respectively.

Higher microbial inhibition activity of **3a-3c** against tested microbes may be due to presence of chloro group in the molecular structure and also due to longer side chain in the compounds**3c**and **5c** made the compounds more potent against microbial growth [11].

CONCLUSION

In summary, the synthesis of alkyl chain bearing chloro and morpholine based *N*-phenothiazine derivatives was carried out. The molecular structure of the synthesized compounds was confirmed by various spectroscopic techniques. The antimicrobial investigations was performed against gram positive bacteria, *B. subtilis* and *S. aureus* and fungal species, *A. flavus* and *A. niger*. The biological results obtained were suggested that chloro based *N*-phenothiazine derivatives were more potent towards inhibition than their morphline analogues. Standard antibacterial and antifungal drugs, Ciprofloxacin and Griseofulvin, respectively, were used for comparison.

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