

Synthesis, characterization and screening of some Schiff bases as potential antimicrobial agents

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Abstract

Schiff bases derived from 4-amino antipyrene were prepared, and IR and NMR spectral analysis characterized their structure. The Schiff bases produced were (1) 4-((4-chlorobenzylidene)amino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-1], (2) 4-((4-hydroxybenzylidene)amino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-2], (3) 1,5-dimethyl-2-phenyl-4-((3-phenylallylidene)amino)-1H-pyrazole-3(2H)-one. [SBS-3], (4) 4-((4-hydroxy-3-methoxybenzylidene)amino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-4], (5) 4-(benzylideneamino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-5], (6) 4-((furan-2-ylmethelene)amino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-6] and (7) 4-((4-methoxybenzylidene)amino)-1-5 dimethyl-1H-pyrazole-3(2H)-one. [SBS-7]. The antibacterial activity was studied against *S. subfava* NCIM 2178, *B. megaterium* ATCC 9885, *P. pseudoalcaligenes* ATCC 17440, *P. vulgaris* NCTC 8313, *C. freundii* ATCC 10787 and *E. aerogenes* ATCC 13048. The antibacterial activity was done using Agar Ditch method. The antibacterial activity was evaluated in two polar solvents DMSO and DMF. A differential effect of the compounds extracted in a particular solvent (DMSO/DMF) inhibited different bacteria to a different level. It supports the earlier conclusion that antibacterial activity is dependent on molecular structure of the compound, solvent used and the bacterial strain under consideration. In the present work, 4-amino antipyrene as central ligand, cinnamaldehyde as side chain and DMF as a solvent appeared to be best in inhibiting the studied bacterial strains.

Keywords: Schiff bases, 4-amino antipyrene, antibacterial activity, DMSO, DMF, drug designing

Introduction

The molecular manipulation of promising lead compounds is still a major line of approach to new drugs. Molecular manipulations involves the efforts to combine the separate groups of similar activity into one compound, and making structural changes into the compound leading to changes in the biological activity.

Schiff bases are considered as very important class of organic compounds which have wide applications such as dyes, liquid crystals, corrosion inhibitors, complexing agents etc.(1-6). Further, Schiff bases have been reported to demonstrate a wide range of pharmacological activities which include antibacterial, antifungal, anti-HIV, antitumor, anti-inflammatory, antipyretic, etc. (7-16).

A variety of Schiff bases have been synthesized from various compounds, the main aim being in search of new lead molecules. Sahu *et al.* (17) synthesized Schiff bases from 2-hydroxy-naphthaldehyde with 2-amino-benzothiazole and evaluated its fungi toxicity. Fioravanti *et al.* (18) observed antibacterial and antifungal activity in few Schiff bases derived from *N*-heteroaryl benzylamines. Shetye *et al.* (19) have reported the synthesis of Schiff bases derived from 3-phenyl salicylaldehyde and studied their biological properties. Khare and Mishra (20) prepared Schiff bases by using 2-amino pyridine and 2-furfuraldehyde. Saghatforoush *et al.* (21) have prepared tetradentate Schiff bases derived from amino thio ether pyridine and salicylaldehyde derivatives. Schiff bases from 4-amino-*N*-carbamimidoyl benzene sulfonamide have been synthesized by Singh *et al.* (22) and their antimicrobial activity has also been studied. Amanullah *et al.* (23) synthesized some novel Schiff bases and studied their cytotoxic, antibacterial activity and physico-chemical properties. Some new heterocyclic Schiff bases have also been synthesized by Deshpande *et al.* (24). Parekh *et al.* (25) synthesized some Schiff bases from 4-amino benzoic acid and studied their antibacterial activity. Vaghasiya *et al.* (26) derived Schiff bases from 4-aminoantipyrine and vanillin and evaluated their potential as antibacterial agents against some Gram positive and Gram negative bacterial strains.

Considering the wide range of biological activities of Schiff bases, in the present work, some new Schiff bases were synthesized from 4-amino antipyrine with different side chains and evaluated their potency as anti bacterial agents.

Experimental

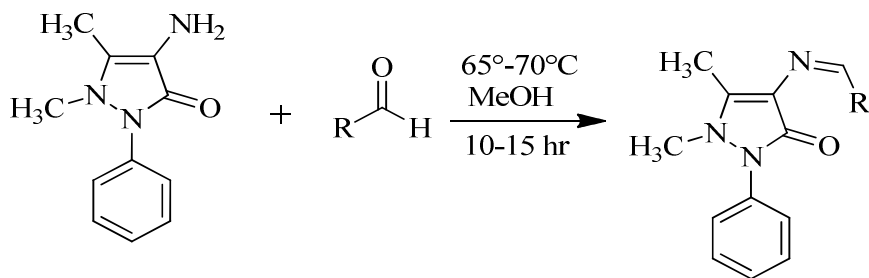
The following Schiff bases have been synthesized:

- (1) 4-((4-chlorobenzylidene)amino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS1]
- (2) 4-((4-hydroxybenzylidene)amino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS2]
- (3) 1,5-dimethyl-2-phenyl-4-((3-phenylallylidene)amino)-1H-pyrazole-3(2H)-one. [SBS3]
- (4) 4-((4-hydroxy-3-methoxybenzylidene)amino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS4]
- (5) 4-(benzylideneamino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS5]
- (6) 4-((furan-2-ylmethelene)amino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS6]
- (7) 4-((4-methoxybenzylidene)amino)-1,5-dimethyl-1H-pyrazole-3(2H)-one. [SBS7]

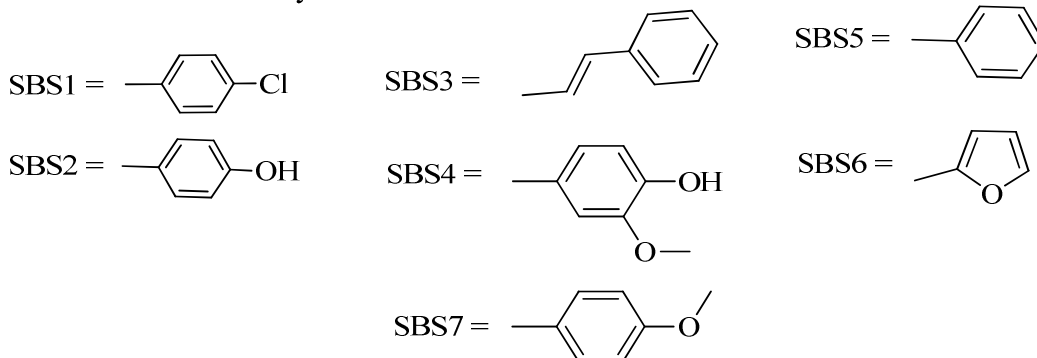
Experimental procedure:

Equimolar mixture of 4-amino antipyrine and different aldehydes were dissolved in methanol and the mixture was refluxed for 10-15 hours at 65-70 °C. The mixture was then poured on crushed ice, filtered and dried.

Synthesis Scheme:



Where R= Different aryl substitution.



Antibacterial activity

The *in vitro* antibacterial activity of the synthesized compounds was evaluated against two Gram positive bacteria (*B. megaterium* ATCC 9885 and *S. subfava* NCIM 2178) and four Gram negative bacteria (*C. freundii* ATCC 10787, *P. vulgaris* NCTC 8313, *P. pseudoalcaligenes* ATCC 17440 and *E. aerogenes* ATCC 13048). The antibacterial activity was done by Agar well method using Mueller Hinton Agar No.2 as the nutrient medium (27). Stock solutions (10 mg/ml) were prepared by dissolving the compound in DMSO or DMF. The bacterial strains were activated by inoculating a loop full of test strain in 25ml of N-broth and the same was incubated for 24h in an incubator at 37 °C. 0.2 ml of the activated strain was inoculated in Mueller Hinton Agar. Mueller Hinton Agar kept at 45°C was then poured in the Petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 24 h at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain and each solvent, where pure solvent was inoculated into the well. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds.

Results and Conclusions

Seven compounds were synthesized and IR and NMR spectral data analysis confirmed their molecular structure. Their data are given below:

SBS1: $^1\text{H NMR}$ (δppm): 2.50 (3H, C-CH₃), 3.18-3.19 (3H, N-CH₃), 7.26-7.50 (9H, Ar-H), 8.19-8.22 (1H, -N=CH).

IR (KBr, cm^{-1}): 3051 (Ar, C-H str.), 2951 (C-H str. (asym)), 2866 (C-H str. (sym)), 1710(C=O str.), 1627 (C=N str.), 1519 (aromatic C=C str.), 1474 (alkane C-H def. (asym.)), 850(mono sub. Aryl ring), 738(C-Cl str.).

Mass (m/e) : 326, 290, 234, 216, 188,121, 107.

SBS2: $^1\text{H NMR}$ (δppm):- 2.42 (3H, C-CH₃), 3.16 (3H, N-CH₃), 4.58(1H, OH), 6.67-7.52 (9H, Ar-H), 8.84(1H, -N=CH)

IR (KBr, cm^{-1}): 3630 (OH str), 3030 (Ar, C-H str.), 2954 (C-H str. (asym)), 2871 (C-H str. (sym)), 1714(C=O str.), 1638 (C=N str.), 1555 (aromatic C=C str.), 1479 (alkane C-H def. (asym.)), 847(mono sub. aryl ring).

Mass (m/e): 308, 277, 217, 199, 188,121, 107.

SBS3: ^1H NMR (δ ppm): 2.45 (3H, C-CH₃), 3.14 (3H, N-CH₃), 5.58-5.62(2H, CH=CH), 7.45-8.2(9H, Ar-H), 8.32(1H, -N=CH).

IR (KBr, cm^{-1}): 3058 (Ar, C-H str.), 3021(CH=CH str), 2941 (C-H str. (asym)), 2869 (C-H str. (sym)), 1717(C=O str.), 1619 (C=N str.), 1519 (aromatic C=C str.), 1477 (alkane C-H def. (asym.)), 849 (mono sub. aryl ring),

Mass (m/e): 318, 289, 225, 203, 188,167, 121, 107.

SBS4: ^1H NMR (δ ppm): 2.46 (3H, C-CH₃), 3.10(3H, N-CH₃), 3.74(3H, OCH₃), 5.4(1H, OH), 6.91-7.31 (8H, Ar- H), 9.6(1H, N=CH).

IR (KBr, cm^{-1}): 3629(O-H str), 3034(Ar, C-H str), 2944(C-H str), 1699 (C=O str), 1540(Ar, C=C str), 1620(C=N str), 1497(Ar, C=C str), 1479(C-H ben), 1161 (C-O-C str.), 1053(C-O str), 845 (mono sub. aryl ring),

Mass (m/e): 338, 307, 322, 202, 188,139, 121, 107.

SBS5: ^1H NMR (δ ppm): 2.49 (3H,C-CH₃), 3.15 (3H, N-CH₃), 7.39-7.66 (10H, Ar-H), 9.76 (1H, N=CH).

IR (KBr, cm^{-1}): 3071 (Ar, C-H str), 2943(C-Hstr), 2867(C-H str), 1610(Ar, C=C str), 1642(C=N str.), 1519(Ar, C=C str), 1512(Ar, C=C str), 1490 (C-H ben), 1427(C-H ben), 1377(C-H ben), 1265(C-Cstr), 844(mono sub. aryl ring).

Mass (m/e): 292, 276, 202, 188, 171, 121, 107.

SBS6: ^1H NMR (δ ppm): 2.47 (3H, C-CH₃), 3.45 (3H, N-CH₃), 6.46-6.56: (8H, Ar-H), 7.45: (1H, N=CH).

IR (KBr, cm^{-1}): 3028(Ar, C-H str), 2933(C-H str), 1725 (C=O str), 1545(Ar, C=C str), 1629(C=N str), 1507(Ar, C=C str), 1480(C-H ben), 1070(C-O str), 841 (mono sub. aryl ring),

Mass (m/e): 282, 265, 202, 188, 121.

SBS7: ^1H NMR (δ ppm): 2.46 (3H, C-CH₃), 3.10 (3H, N-CH₃) 3.65 (3H, O-CH₃), 6.44-7.69 (9H, Ar-H), 9.66 (1H, N=CH).

IR (KBr, cm⁻¹): 3034(Ar, C-H str), 2940(C-H str), 1722 (C=O str), 1661(C=N str), 1530(Ar, C=C str), 1497(Ar, C=C str), 1459(C-H ben), 1160(C-O-C str), 850 (mono sub. aryl ring)

Mass (m/e): 322, 307, 290, 230, 213, 202, 188, 134, 121.

The molecular formula, molecular weight, melting point, % yield and R_f values along with the solvent systems of 7 Schiff bases are given in table 1.

Table 1: Analytical and physical data of the synthesized compounds\

Compound Code	Molecular Formula	Molecular Weight (gm/Mol)	M.P ^o C	% Yield	R _f Value [*]
SBS-1	C ₁₈ H ₁₆ N ₃ OCl	325.5	193	63	0.62
SBS-2	C ₁₈ H ₁₇ N ₃ O ₂	307	197	59	0.64
SBS-3	C ₂₀ H ₁₉ N ₃ O	317	98	52	0.61
SBS-4	C ₁₉ H ₁₉ N ₃ O ₃	337	194	67	0.30
SBS-5	C ₁₈ H ₁₇ N ₃ O ₂	291	163	56	0.60
SBS-6	C ₁₆ H ₁₅ N ₃ O ₂	281	216	58	0.38
SBS-7	C ₁₉ H ₁₉ N ₃ O ₂	321	159	59	0.48

* Acetone: Benzene (1:9)

The antibacterial activity of seven synthetic compounds derived from 4-amino antipyrene in DMSO and DMF against *B. megaterium* and *S. subfava* are shown in Fig.1. Maximum antibacterial activity was shown by SBS3 in both the solvents against *B. megatarium*; SBS3 showed more activity in DMF than in DMSO. SBS1, and SBS2 showed slight activity only in DMF while SBS5 and SBS6 showed activity only in DMSO against this bacterial strain. SBS4 showed some activity in DMSO and very negligible activity in DMF. And lastly SBS7 showed no activity against *B. megatarium* in either of the solvent (Fig.1).

The antibacterial activity of the seven synthesized compounds against *S. subfava* are shown in Fig. 1. All the compounds showed quite less activity against this Gram positive strain in both the solvents as compared to *B. megatarium*. SBS3 showed maximum activity in both the solvents against this bacterial strain also. SBS1, SBS2 and SBS7 showed slight activity only in DMF and rest of the compounds did not show any activity in both the solvent against this strain (Fig.1). The differential response of the synthesized compounds is because of the difference in their structure and also the solvent in which they are dissolved. All the seven compounds have 4-amino antipyrine as their central ligand and the side chains are different. In SBS1 it is chlorobenzaldehyde, in SBS2 it is hydroxy benzaldehyde, in SBS3 it is cinnamaldehyde, in SBS4 it is vanillin, in SBS5 it is benzaldehyde, in SBS6 it is furfuraldehyde and in SBS7 it is p-anisaldehyde.

The antibacterial activity against the Gram negative bacteria *C. freundii* and *P. vulgaris* in both polar solvents DMSO and DMF are shown in Fig. 2. Here an entirely different trend was observed. Only three compounds i.e. SBS1, SBS2 and SBS3 in DMF showed antibacterial activity against *C. freundii* and maximum activity was shown by SBS3. All the other compounds in both the solvents did not show any activity against *C. freundii* (Fig.2). All the synthesized seven compounds showed antibacterial activity against *P. vulgaris* in DMSO while none of the compounds exhibited antibacterial activity in DMF. Maximum antibacterial activity was shown by SBS1, closely followed by SBS2 and SBS3. Minimum activity was shown by SBS6 and SBS7 (Fig.2). This again firmly proves and confirms our earlier conclusion that there is a direct and strong relation between structures of the compound, solvent in which it is extracted and the bacterial strain against which it is studied.

The antibacterial activity against *P. pseudoalkaligenes* and *E. aerogenes* are shown in Fig. 3. All the seven compounds synthesized from 4-aminoantipyrine extracted in DMSO could not inhibit the growth of either of these two Gram negative bacteria. The compounds extracted in DMF inhibited both these bacteria but a differential effect was envisaged. All the compounds extracted in DMF could inhibit *P. pseudoalkaligenes* but each compound had a different level of inhibition. Maximum inhibitory activity was shown by SBS4 and SBS5 followed by SBS3, SBS6 and SBS7 respectively. Minimum activity was by SBS1 and SBS2. In *E.*

aerogenes, only SBS3 showed some inhibition while all the other compounds showed no activity.

In the present work, amongst the seven synthesized compounds and the two solvents used, the best activity was shown by SBS3 in DMF solvent. Hence, it can be concluded that cinnamaldehyde as side chain with 4-amino antipyrine as central ligand and DMF as a solvent are the best in inhibiting the studied bacterial strains. This once again proves the earlier conclusion that antibacterial activity is dependent on molecular structure of the compound, solvent used and the bacterial strains under consideration (25-26). Such screening of various organic compounds and identifying active agents is the need of the hour; because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

For initial screening of synthesized compounds, a higher concentration is used to find out the most potent compound. Then, the potent compound is further evaluated for its MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values. Work in this direction is in progress. In the present work, the potent compound is SBS3.

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Fig. 1

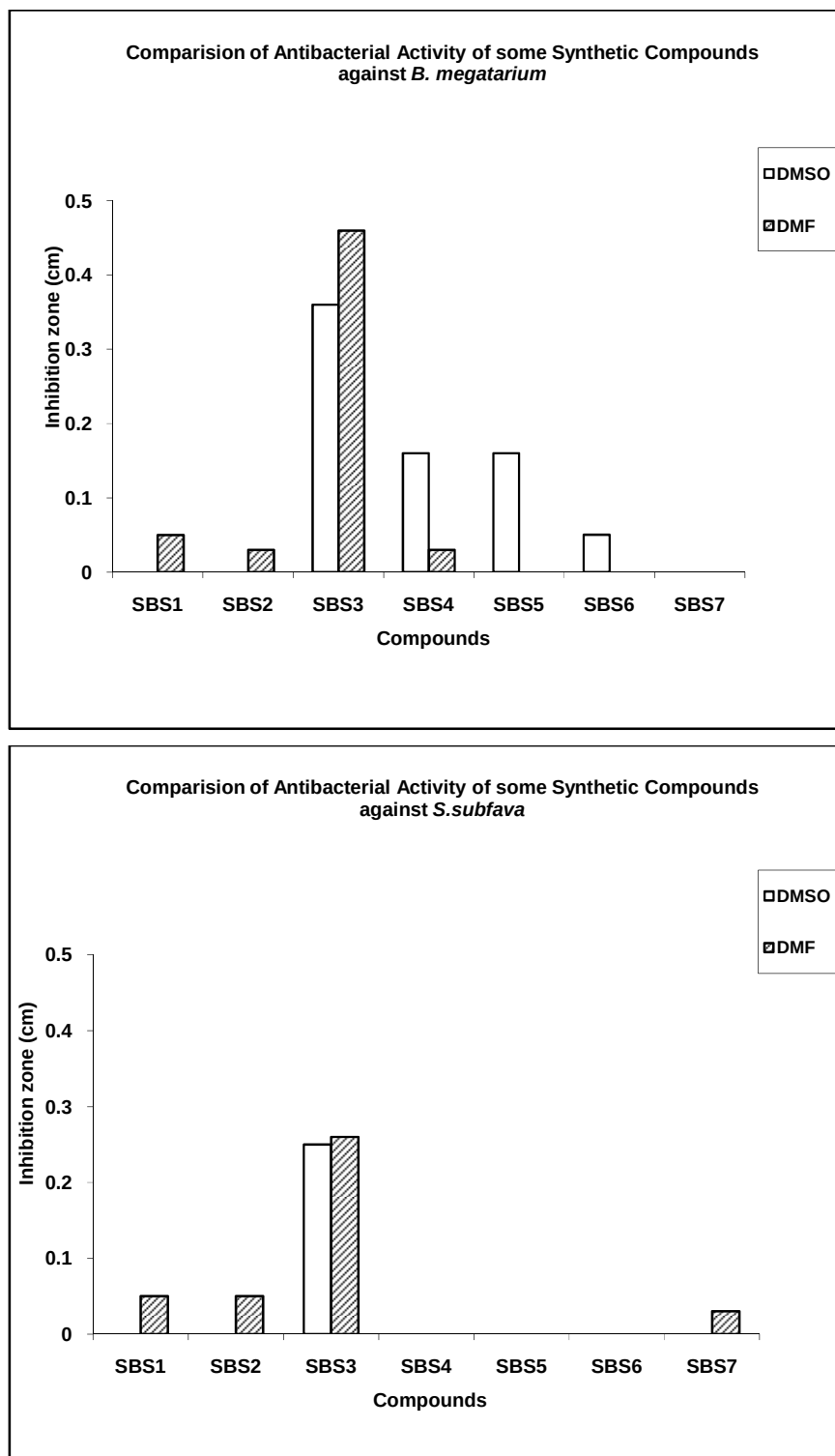


Fig: 2

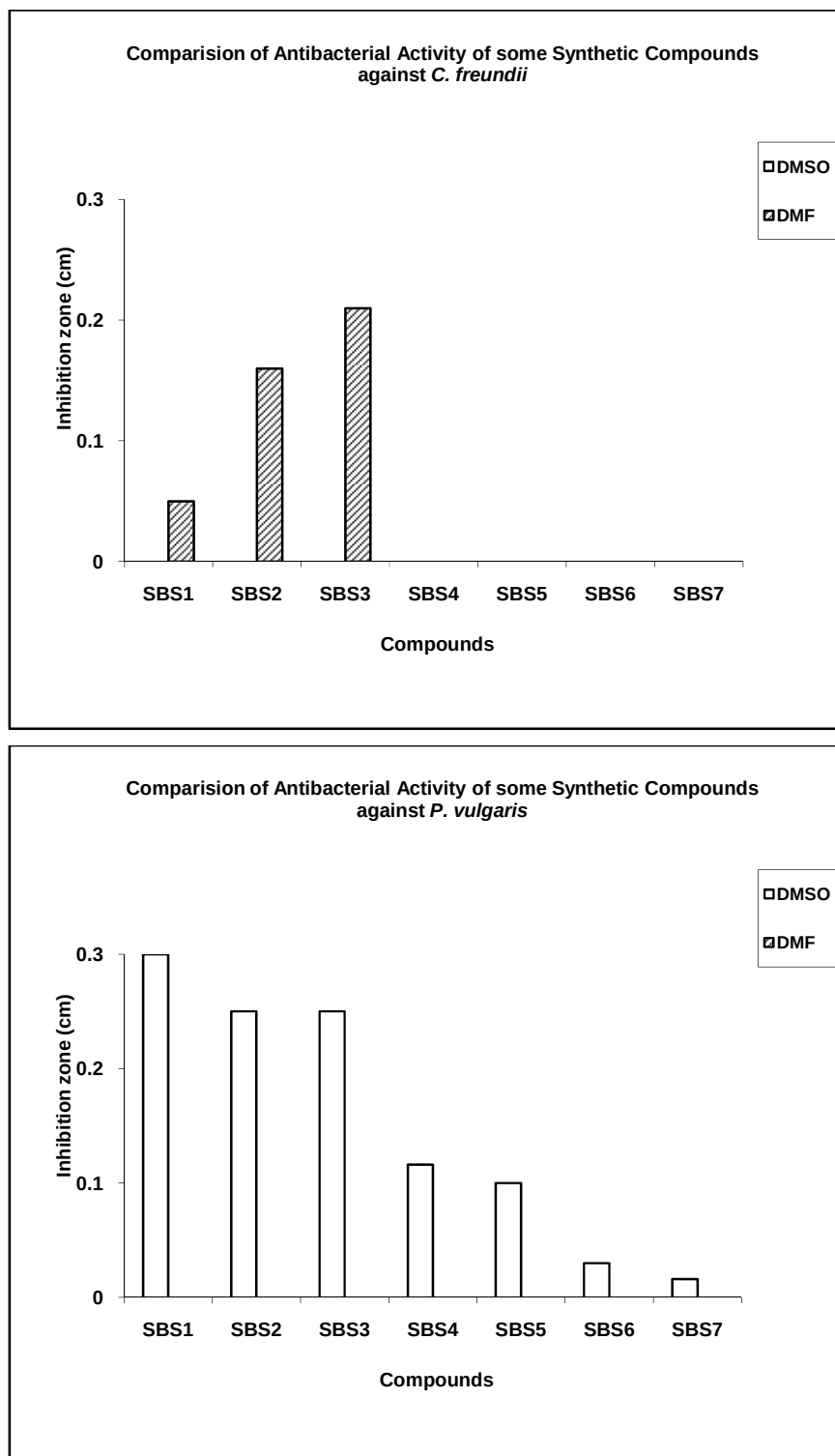


Fig: 3

