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**Research Article**

# Synthesis and Investigation of Antibacterial activity of Some Schiff bases

**Shipra Baluja[1*] and Sumitra Chanda[2]**

[1]Physical Chemistry Laboratory, Department of Chemistry, Phytochemical, Pharmacological and Microbiological Laboratory, Rajkot 360 005, India

[2]Department of Biosciences, Saurashtra University, Rajkot 360 005, India

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***ABSTRACT***

***Eight Schiff bases were synthesized from 4-amino benzoic acid and were characterized by IR and NMR spectral analysis. In all 8 compounds were synthesized viz. (1) 4-[(4-Methoxy-benzylidene)-imino]-benzoic acid, (2) 4-(Benzylidene-imino)-benzoic acid, (3) 4-[(2-chloro-benzylidene)-imino]-benzoic acid, (4) 4-[(Furan-2-ylmethylene)-imino]-benzoic acid, (5) 4-(3-Phenyl-allylideneimino)-benzoic acid, (6) 4-[(2-Hydroxy-benzylidene)-imino] -benzoic acid, (7) 4-[(4-Hydroxy-3-methoxy-benzylidene)-imino]-benzoic acid and (8) 4-[(3-Nitro-benzylidene)-imino]-benzoic acid. Antibacterial activities of these synthesized Schiff bases have been tested against six bacterial strains. The studied bacterial strains are S. aureus ATCC 25923, B. cereus ATCC 11778, P. mirabilis NCIM 2241, P. aeruginosa ATCC 27853, S. typhimurium ATCC 23564 and A. fecalis ATCC 8750. The antibacterial activity was done by Agar Ditch method. The solvents used for studying antibacterial activity were 1, 4-dioxan (non-polar) and DMF (polar, Dimethylformamide). A differential effect of the compounds was found in the bacterial strains investigated and the solvents used, suggesting once again that the antibacterial activity is dependent on the molecular structure of the compound, solvent used and the bacterial strains under consideration.***

## INTRODUCTION

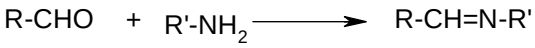
Schiff bases are studied extensively because of their high potential chemical permutation. They are known to be useful in perfumery[1], as corrosion inhibitor[2], as complexing agents[3] and as intermediate in the synthesis of various compounds[4-8]. These bases have applications in various other fields also[9-12]. Further, they are known to possess broad spectrum of biological activity such as antitumer[13], antifungal[14], antimicrobial[15], insecticidal[16], anti-inflammatory[17] etc. In fact, they are of biological, pharmaceutical and

of analytical interest. Schiff bases are characterized by the –N=CH- (imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological systems[18,19]. Metzler *et al*.[20] have studied the Schiff bases derived from pyridoxal and threonine as an important intermediate of many biochemical reactions. Schiff bases derived from triazoles, substituted triazoles and phenyl derivatives show antimicrobial activity[21-23]. Mohan and Saravanan [24] have also reported antibacterial activity of some Schiff bases involving 2-$NH_2$-3- (-N-tolylcarboxamide)-4, 5 trimethelene thiophenes. Many Schiff bases have been known to be medicinally important and are used to design novel medicinal agents[25,26].

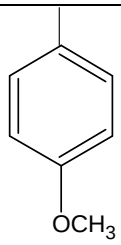
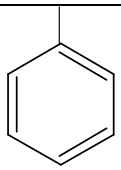
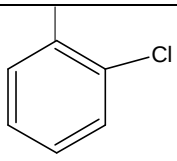
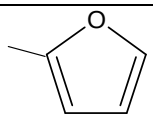
Considering the above, the aim of present work was to synthesize some Schiff bases from 4-iminobenzoic acid with different side chains and to evaluate their potency as anti bacterial agents. The effect of substitution on its inhibition will also be compared.

## MATERIAL AND METHODS

**Synthesis of Schiff bases:** The requisite amount of corresponding aldehyde was dissolved in 200 ml methanol. 0.1 M of p-imino benzoic acid and few drops of glacial acetic acid were added to it and the mixture was refluxed for 10-12 h at 70-80$^o$ in water bath. The resulting solution was cooled to room temperature, and then poured over crushed ice with constant stirring. The precipitate was filtered and washed with sodium bisulfite solution to remove excess of aldehyde. The product was crystallized from hot methanol and dried.




In these reactions for PB1-8, R' is benzoic acid, and corresponding R is as given Fig. 1.

| R | Name of the compound | Code |
|---|---|---|
|  | 4-[(4-Methoxy-benzylidene)-imino]-benzoic acid | PB1 |
|  | 4-(Benzylidene-imino)-benzoic acid | PB2 |
|  | 4-[(2-chloro-benzylidene)-imino]-benzoic acid | PB3 |
|  | 4-[(Furan-2-ylmethylene)-imino]-benzoic acid | PB4 |

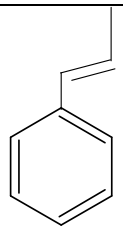
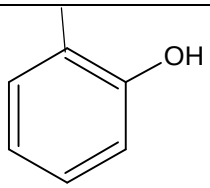
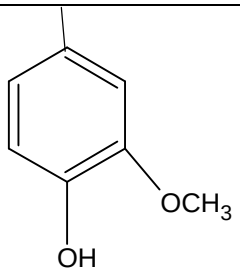
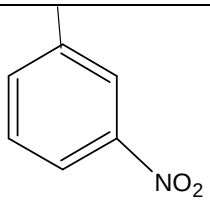
| | | |
|---|---|---|
|  | 4-(3-Phenyl-allylideneimino)-benzoic acid | PB5 |
|   | 4-[(2-Hydroxy-benzylidene)-imino] -benzoic acid | PB6 |
|   | 4-[(4-Hydroxy-3-methoxy-benzylidene)-imino]- benzoic acid | PB7 |
|   | 4-[(3-Nitro-benzylidene)-imino] -benzoic acid | PB8 |

**Fig. 1:** Substituent R, IUPAC names and codes of synthesized compounds.

**Microorganisms:** The bacterial strains studied are identified strains and were obtained from National Chemical Laboratory (NCL), Pune, India. The investigated microorganisms belonged to both gram-positive and gram-negative category.

**Antibacterial activity:** Antibacterial activity was evaluated by agar well diffusion method [27]. A loop full of the given test strain was inoculated in 10 ml of N-broth and was incubated for 24 h in an incubator at 37°C in order to activate bacterial strain. Inoculation of the test strain was done by the pour-plate technique. The activated strain (0.2 ml) was inoculated into the media when it reached 40-45°C temperature. The media was allowed to solidify. After solidification of the media, loop ditch was made in the plates with the help of cup-borer (0.85 cm) and then the synthetic compound (dissolved in DMF/1,4-dioxan) was inoculated into the well. Synthetic compounds were soluble in DMF and 1,4-dioxan and therefore the antibacterial activity is studied using these solvents. The controls were maintained (for each bacterial strain and each solvent), where 0.1 ml of the 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 DISCUSSION

In all, 8 compounds were synthesized and IR and NMR spectral data confirmed their molecular structure. The physical properties tested are given in **Table 1** and the IR and NMR analysis data are given in **Table 2**. The element analysis of eight compounds has also been determined and are given in **Table 3**.

**Table 1**: Compound code, molecular formula, molecular weight, melting point, percentage yield and $R_f$ values along with its solvent system

| Compound Code | Molecular Formula | Molecular Weight (gm/mol) | M.P $^oC$ | % Yield | $R_f$* Value |
|---|---|---|---|---|---|
| PB1 | $C_{15}H_{13}NO_3$ | 255.269 | 186 | 62 | 0.61* |
| PB2 | $C_{14}H_{11}NO_2$ | 225.243 | 170 | 45 | 0.51 |
| PB3 | $C_{14}H_{10}NO_2Cl$ | 256.688 | 217 | 52 | 0.45* |
| PB4 | $C_{12}H_9NO_3$ | 215.205 | >300 | 59 | 0.48 |
| PB5 | $C16H13NO_2$ | 251.28 | 172 | 63 | 0.58 |
| PB6 | $C_{14}H_{11}NO_3$ | 241.24 | 260 | 57 | 0.41* |
| PB7 | $C_{15}H_{13}NO_4$ | 271.268 | 115 | 55 | 0.52 |
| PB8 | $C_{13}H_9N_2O_4$ | 257.22 | 250 | 65 | 0.34 |

* Ethyl acetate + Hexane (2.5 + 7.5)
Acetone + Benzene (4.0 + 6.0)

**Table 2:** IR and NMR data of synthesized compounds

| Compound Code | IR $cm^{-1}$ (KBr) | | | | | | NMR (δ ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | -OH (str.) | C=O (str.) | N=C | C-O-C (str.) | -OH (Bend) | C-Cl* / C-$NO_2$ | $OCH_3$ | Ar-H | N=CH | Ar-OH | COOH |
| **PB1** | **3421** | **1683** | **1617** | **1446** | **1315** | **-** | **3.89** | **6.82-7.84** | **8.40** | **-** | **9.88** |
| **PB2** | **3460** | **1681** | **1602** | **-** | **1315** | **-** | **-** | **6.61-8.04** | **8.48** | **-** | **9.88** |
| **PB3** | **3344** | **1685** | **1608** | **-** | **1315** | **839*** | **-** | **6.62-7.75** | **8.89** | **-** | **10.45** |
| **PB4** | **3352** | **1681** | **1601** | **1388** | **1271** | **-** | **-** | **7.10-7.72** | **8.30** | **-** | **9.48** |
| **PB5** | **3321** | **1674** | **1601** | **-** | **1311** | **-** | **-** | **6.89-8.12** | **8.25** | **-** | **9.81** |
| **PB6** | **3417** | **1681** | **1581** | **-** | **1419** | **-** | **-** | **6.62-8.06** | **8.82** | **10.09** | **10.75** |
| **PB7** | **3367** | **1685** | **1601** | **1396** | **1315** | **1571** | **3.90** | **6.95-8.00** | **8.34** | **9.07** | **10.60** |
| **PB8** | **3315** | **1692** | **1602** | **-** | **1315** | **-** | **-** | **6.68-8.26** | **8.60** | **-** | **10.10** |

**Table 3:** CHN analysis of synthesized compounds.

| S. No. | Compound Code | C | | H | | N | | O | | Cl | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cal. | Obs. | Cal. | Obs. | Cal. | Obs. | Cal. | Obs. | Cal. | Obs. |
| 1 | PB1 | 70.50 | 70.52 | 5.09 | 5.10 | 5.48 | 5.46 | 18.80 | 18.85 | - | |
| 2 | PB2 | 74.59 | 74.60 | 4.88 | 4.90 | 6.22 | 6.25 | 14.21 | 14.25 | - | |
| 3 | PB3 | 65.45 | 65.52 | 3.90 | 3.90 | 5.45 | 5.42 | 12.47 | 12.50 | 13.83 | 13.86 |
| 4 | PB4 | 66.91 | 66.92 | 4.18 | 4.21 | 6.51 | 6.55 | 22.30 | 22.35 | - | |
| 5 | PB5 | 76.41 | 76.45 | 5.17 | 5.19 | 5.57 | 5.60 | 12.73 | 12.77 | - | |
| 6 | PB6 | 69.64 | 69.68 | 4.56 | 4.61 | 5.80 | 5.85 | 19.90 | 19.95 | - | |
| 7 | PB7 | 66.36 | 66.42 | 4.78 | 4.79 | 5.16 | 5.18 | 23.59 | 23.63 | - | |
| 8 | PB8 | 60.65 | 60.69 | 3.50 | 3.51 | 10.89 | 10.94 | 24.88 | 24.90 | - | |

These 8 synthetic compounds and their respective controls produced different inhibition zones against the tested bacterial strains. The controls were deducted from the tested compounds; their effect was noticeably different depending on the type of the solvent used.

The results of antibacterial screening indicate that these Schiff bases show considerable activity against the studied microorganisms. The antibacterial activity against *P. aeruginosa* and *P. mirabilis* are shown in **Fig. 2.**

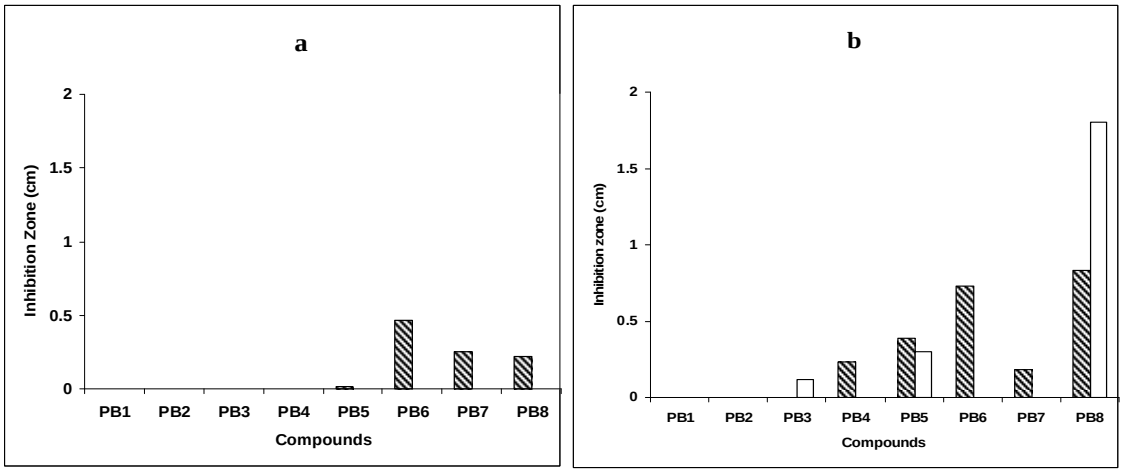



**Fig. 2:** Antibacterial activity of some synthetic compounds against P. aeruginosa (a) and P. mirabilis (b)

(Blank Bar - 1,4-dioxan and Filled bar – DMF)

When 1,4-dioxan was used as a solvent the synthesized compounds failed to show any inhibitory activity against *P. aeruginosa*, while when DMF was used as a solvent some of them viz. PB6-PB8 showed considerable inhibition. On the other hand, the same compounds with both the solvents could inhibit *P. mirabilis* to various level of inhibition. The compound PB6 in DMF showed maximum inhibition in *P. aeruginosa* followed by PB7 and PB8, while when *P. mirabilis* considered maximum inhibition was showed by PB5 and PB3 followed by PB6 and PB8. Other compounds showed negligible levels. The activity was almost similar with both the solvents. This differential response of the compounds is because of the structural differences amongst them. In all eight compounds, the central molecule is 4-iminobenzoic acid while the attachment are different. From PB1 to PB8 the attachments are as follows:- p-anisaldehyde, benzaldehyde, o-chlorobenzaldehyde, furfuraldehyde, cinnamaldehyde, salisaldehyde, vanillin, m-nitrobenzaldehyde. It can be clearly seen that the group or the molecule that is attached to the

central ligand play an important role in inhibiting the bacteria. The inhibition of bacterial growth depends on the solubility of the compounds in particular solvent, its diffusion capacity and penetration into the bacterial cell wall.

The inhibitory activity of the synthesized Schiff bases in DMF and 1,4-dioxan against *S. typhimurium* and *A. fecalis* shown in **Fig. 3**.

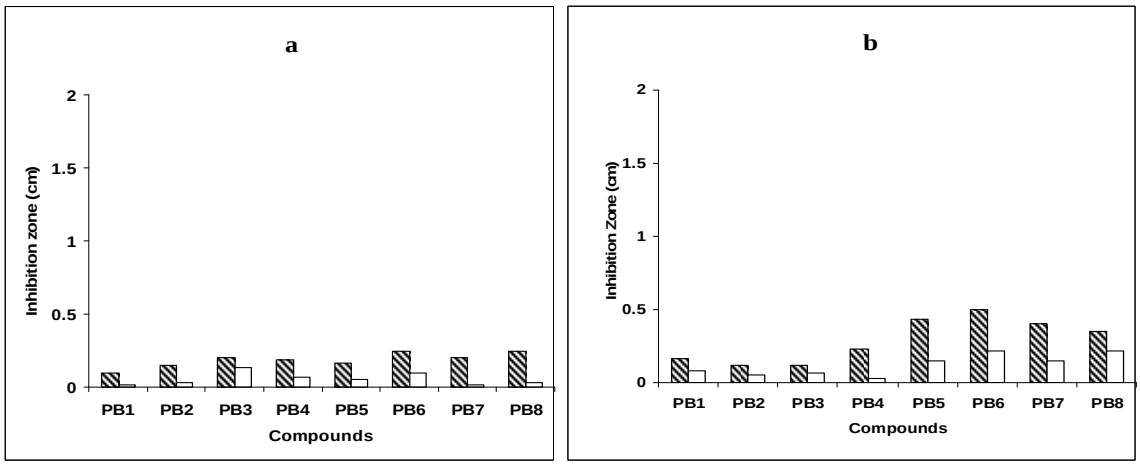



**Fig. 3:** Antibacterial activity of some synthetic compounds against *S. typhimurium* (a) and *A. fecalis* (b) (Blank Bar - 1,4-dioxan and Filled bar – DMF)

The activity in both the solvents was more in *A. fecalis* as compared to *S. typhimurium* except PB2 and PB3. The solvent 1,4-dioxan gave considerably more inhibition than DMF. In *S. typhimurium* all the eight compounds in DMF almost showed similar antibacterial activity though activity of compounds PB6 and PB8 was maximum. The compounds in 1,4-dioxan gave slight inhibition against these bacteria. In *A. fecalis*, maximum inhibitory activity shown by PB6 followed by PB5, PB7 and PB8 in DMF, while the other compounds showed slightly less inhibitory activity. The compounds in 1,4-dioxan showed a similar trend.

The antibacterial activity of synthesized compounds against gram-positive bacteria *S. aureus* and *B. cereus* in DMF and 1,4-dioxan are shown in **Fig. 4**.

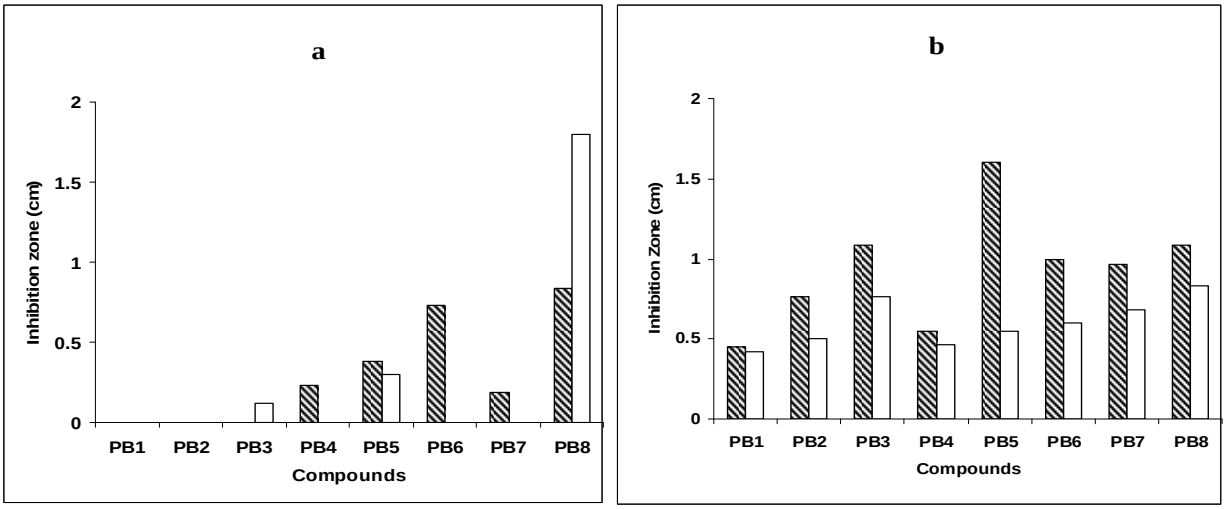



**Fig. 4:** Antibacterial activity of some synthetic compounds against S. aureus (a) and B. cereus (b) (Blank Bar - 1,4-dioxan and Filled bar – DMF)

The activity was more in *B. cereus* than in *S. aureus*. In both the bacteria, the activity was more in DMF than in 1,4-dioxan. In *S. aureus* only the compound PB8 showed considerable inhibition in 1,4-dioxan,

while in DMF compounds PB8 and PB6 showed maximum activity; the other compounds showed negligible activity. In *B. cereus*, in DMF the maximum inhibitory activity was shown by PB5 followed by PB6-PB8. The other three compounds also showed inhibitory activity but to a lesser degree. The compounds in 1,4-dioxan showed the same trend.

From the present work, it can be concluded that DMF (appears) is better than 1,4-dioxan in inhibiting the studied bacterial strains except PB8 for *S. aureus* and *P. mirabilis*. This is in agreement with our earlier work[28] that the polar solvents may be beneficial in our attempt to search for lead molecules for drug designing. 4-iminobenzoic acid can be used as a central ligand as lead molecule with salicyldehyde and m-nitrobenzaldehyde as side chain.

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*Correspondence Author: **Shipra Baluja;** Physical Chemistry Laboratory, Department of Chemistry[1], Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences2, Saurashtra University, Rajkot 360 005, India