Synthesis and biological evaluation of new aryl substituted Schiff’s bases

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ABSTRACT

Objective: Chemical substances employed to treat various infections caused by various types of microorganism are termed as antimicrobials and natural chemical compounds produced by specific types of bacteria are termed as antibiotics. Unlimited use of antibiotics in humans and animals and in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. Various attempts have been adopted to cope with the resistance problem and enhance the activity, or broaden the spectrum of drugs. Based on structure-activity relationship synthesis of new compounds has been one of the best approaches for better results. It has been demonstrated that Schiff base of some leading molecules and antibiotics possess good potential as more effective and safe drugs. Encouraged by reports on potential of Schiff’s bases as antimicrobial agents and to cope up with the current requirements of developing newer, safer and broad spectrum agents attempts were made to synthesize new Schiff’s bases.

Methods: Our earlier in which structure activity relationship studies revealed that substitution by nitro and amino gp in Schiff’s base moiety resulted in the enhancement of activity. So further attempts were made to extend the series with incorporation of nitro and amino moiety by condensing o,m dinitro substituted acid hydrazide with various nitro/amin substituted benzaldehydes for increasing their antimicrobial potential.

Results: Synthesized compounds were characterized on the basis of spectral studies (like UV, IR, and NMR). All the synthesized derivatives were screened further for their antibacterial effect. All the synthesized derivatives were screened further for their antibacterial effect.

Conclusions: Highest activity was observed in the derivative with nitro substitution in both the aryl rings.

Keywords: Antibiotics, Schiff’s base, Hydrazides
Introduction

The antimicrobial agents are broadly categorized as respectively in antibiotics and antibacterial. Natural chemical compounds produced by specific types of bacteria are termed as antibiotics and that of synthetic origin are termed as antibacterial agents wherein the product of bacterial process is modified to prepare a compound having desired biological action are semi-synthetic antibiotics. However, some of the antibiotics originally produced by microorganisms can be totally synthesized by chemical methods. They are generally referred to as synthetic antibiotics in order to distinguish them from the synthetic antimicrobial agents. Antimicrobial therapy owes its initiation from penicillin in 1929 and sulfonamides in 1935 followed by isolation of streptomycin, chloramphenicol and tetracycline. Antibiotics must possess selective toxicity for a particular targeted microorganism and be safe for human beings. However for a drug to be active it is must that it should be toxic. In order to qualify as a useful drug substance the efficacy must dominate to a significant extent over toxicity.

Unlimited use of antibiotics in humans and animals and in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. Various attempts have been adopted to cope with the resistance problem and enhance the activity, or broaden the spectrum of drugs. Based on structure-activity relationship synthesis of new compounds has been one of the best approaches for better results. It has been demonstrated that Schiff base of some leading molecules and antibiotics possess good potential as more effective and safe drugs. Encouraged by reports on potential of Schiff’s bases as antimicrobial agents and to cope up with the current requirements of developing newer,

![Chemical Structures](image)

| S.No | R₁          | R₂          |
|------|-------------|-------------|
| 1    | o,m Nitro   | -H          |
| 2    | o,m Nitro   | p NH₂-o NO₂ |
| 3    | o,m Nitro   | -P-NH₃ m -NO₂ |
| 4    | o,m Nitro   | o-NH₂-o NO₂ |
| 5    | o,m Nitro   | o- NH₂ p NO₂ |
| 6    | o,m Nitro   | p NO₂ o- NO₂ |
| 7    | o,m Nitro   | p NO₂, m - NO₂ |
safer and broad spectrum agents attempts were made to synthesize new Schiff’s bases.

In continuation to our earlier work in which structure activity relationship studies revealed that substitution by nitro and amino group in Schiff’s base moiety resulted in the enhancement of activity. So further attempts were made to extend the series with incorporation of nitro and amino moiety for increasing their antimicrobial potential. Synthesized compounds were characterized on the basis of spectral studies (like UV, IR, and NMR). All the synthesized derivatives were screened further for their antibacterial effect.

### Materials and Methods

A.R Grade and L.R Grade chemicals were used in the present work and purchased from Rankem, CDH labs and Himedia. Thiel’s tubes melting point apparatus was used to record melting point of the synthesized compounds in the capillary tubes Purity of the compound was checked by thin layer chromatography using silica gel G as stationary phase and various combinations of solvents as mobile phase mixture of Petroleum ether and ethyl acetate in 1:1 ratio. The spot resolved were visualized by using iodine chamber. The compounds are synthesized by conventional method (Figure 1). The technique employed for the characterization of the

| Compound | UV ($\lambda_{\text{max}}$) nm | IR KBR ($\ddot{\text{v}}$ cm$^{-1}$) | $^1$H NMR($\delta$) |
|----------|------------------------------|-----------------------------------|-------------------|
| 1        | 303                          | 3100 (C-H)                         | 7.96-7.94 (m 3H, o,m dinitro ArH ) 7.23, (m 5H Ar H) |
|          |                              | 1603 (C=C)                         | 6.82 (brs 1HN=CH)   |
|          |                              | 1695(C=O)                          |                   |
|          |                              | 1367(C-N)                          |                   |
| 2        | 342                          | 3396 (NH)                          | 8.04-7.97 (m3H, o,m dinitro ArH) 7.65 (m 3H Ar H), |
|          |                              | 2958 (C-H)                         | 6.85 (brs, 1H, N=CH) & 3.29 brs s2H,NH) |
|          |                              | 1689 (CONH)                        |                   |
|          |                              | 1518 (C=C)                         |                   |
| 3        | 332                          | 3485 (NH$_2$)                      | 8.0-7.95 (m,3H o,m dinitroArH), and 7.55 m 3H Ar H,6.82 ((brs, 1H, N=CH) |
|          |                              | 3126 (C-H)                         | 3.05 (brs,2H,NH)   |
|          |                              | 1691 (CONH)                        |                   |
|          |                              | 1529 (C=C)                         |                   |
| 4        | 352                          | 3395 (NH$_2$)                      | 7.93-7:7(m,3H o,m dinitroArH),7.56-7.54 |
|          |                              | 1707 (CONH)                        | (m, 3HArH) |
|          |                              | 2952 (C-H)                         | 6.58 ( brs,1H, N=CH) |
|          |                              | 1602 (C=C)                         | 2.62 (brs2H,NH)    |
| 5        | 326                          | 3300 (NH)                          | 8.0-7.98(m,3H o,m dinitroArH) 7.46-7.43 |
|          |                              | 3391 (NH)                         | (m, 3HArH),6.83(brs, 1H, N=CH) & 3.05 |
|          |                              | 3102 (C-H)                         | (brs, 2H, NH)      |
|          |                              | 1705 (CONH)                        |                   |
|          |                              | 1603 (C=C)                         |                   |
| 6        | 327                          | 3150 (NH)                          | 7.97-7.95 (m,3H o,m dinitroArH, 7.54-7.52 |
|          |                              | 3100 (C-H)                         | (m, 3H,ArH),6.86(brs,1H,N=CH) & 3.05 |
|          |                              | 1691 (CONH)                        | (brs 2H, NH)      |
|          |                              | 1650 (C=C)                         |                   |
| 7        | 367                          | 3100(C-H)                          | 8.10-8.07 (m,3H,ArH) |
|          |                              | 1657 (C=C)                         | 7.97-7.95(m,3H,o,m dinitroArH), 6.91 (brs, |
|          |                              | 1695(C=O)                          | 1H, N=CH)         |
|          |                              | 1367(C-N)                          |                   |

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Table 2: Data of antimicrobial activities of synthesized compounds inhibition of zone diameter (mm).

| Compounds                        | Salmonella typhimurium | Shigella Sonnei | Staphylococcus aureus | Bacillus cereus |
|----------------------------------|------------------------|-----------------|-----------------------|-----------------|
| 1                                | 11                     | 9               | 12                    | 9               |
| 2                                | 13                     | 8               | 15                    | 10              |
| 3                                | 10                     | 10              | 15                    | 8               |
| 4                                | 7                      | 14              | 11                    | 10              |
| 5                                | 12                     | 9               | 10                    | 14              |
| 6                                | 19                     | 17              | 18                    | 19              |
| 7                                | 22                     | 20              | 21                    | 20              |
| Ciprofloxacin (200 µg/disc)      | 24                     | 22              | 21                    | 24              |
| Norfloxacin (c)                  | 24                     | 23              | 22                    | 23              |
| Dimethyl formamide (DMF)         | -                      | -               | -                     | -               |

synthesized compound was UV, IR Spectra and ¹HNMR Spectroscopy. UV was recorded in solvents as per solubility of the compounds using Shimadzu spectrometer and values are recorded as (λ max) nm The IR spectra of synthesized compound were recorded on a Fourier transform IR spectrometer (model Shimadzu 8400s) in the range of 400-4000 using KBR Pallets and the value of ν max is written in cm⁻¹ while ¹HNMR is recorded on Perkin FT NMR spectrophotometer and chemical shift is expressed in δ (Table 1).

**General procedure for synthesis of compounds**

The general procedure for synthesis of compounds takes place in following steps:

(a) **Synthesis of methyl ester of o,m dinitro benzoic acid (i)**

To 1 mole of o,m dinitro benzoic acid in methanol was added few drops of sulphuric acid and refluxed the mixture under anhydrous condition for 6 hours. Reaction mixture was cooled and solvent was distilled off. Residue was collected and recrystallised using ethanol as solvent.

(b) **Synthesis of o,m dinitro benzoic acid hydrazide (iii) from methyl ester o,m dinitro benzoic acid (ii)**

To one mole of methyl ester of o,m dinitro benzoic acid (i) in methanol was added one mole of hydrazine hydrate and refluxed the mixture for 7 hours under anhydrous condition. The reaction was monitored by TLC using various ratios of chloroform, petroleum ether and ethyl acetate. Spots were observed in iodine vapors. Residue product was collected after removal of solvent on water bath.

(c) **Synthesis of 2-[(Substitutedphenylhydrazinylidene) Substituted o,m dinitro benzoyl] aniline (iv1-7) from of o,m dinitro benzoic acid hydrazide (iii)**

To solution of acid hydrazide 2 ml was added 1.58 gms of anhydrous sodium acetate in 20 ml of water and 1.5 ml substituted benzaldehyde with continuous shaking till solid begins to separate out. Solid so obtained was filtered and recrystalized using ethanol.

**Antibacterial activity**

All synthesized compounds 2-[(Substitutedphenylhydrazinylidene) substituted o,m dinitro benzoyl] aniline (iv 1-7) were tested for their in vitro antibacterial activity against gram positive bacteria by using Mueller-Hinton agar medium (HI-Media laboratories, India) was employed to study the preliminary antibacterial activity.⁶⁻⁹
**Paper disc diffusion method**

The sterilized (autoclaved at 120°C for 30 minutes) medium (40-50°C) was inoculated (1 ml/ml of the media) with the suspension of microorganisms and poured in to petri dish to give a depth of 3-4 mm the paper impregnated with the test compounds (200 µg/ml in dimethylformamide) was placed on solidify media. All plates were incubated for 1 h at room temperature at 37°C for 24 h for antibacterial activities respectively. Ciprofloxacin and Norfloxacin were used as standards for antibacterial activity. The observed Zone of inhibition is represented in the Table 2.

**Results and Discussion**

Over, the past few decades, intensive and haphazard use of antibiotics agents has lead to drug resistant microbial pathogens necessitating the need for research for new antimicrobial agents with reduce resistant to pathogens and for better biological activity. As stated Schiff’s bases are reported to have broad-spectrum antimicrobial, antifungal, ant tubercular, anticancer and anti-HIV of the clinicians. In continuation to our earlier in which structure activity relationship studies revealed that substitution by nitro and amino gp in Schiff’s base moiety resulted in the enhancement of activity. So further attempts were made to extend the series with incorporation of nitro and amino moiety for increasing their antimicrobial potential. Synthesized compounds were characterized on the basis of spectral studies (like UV, IR, and NMR). UV \( \lambda_{max} \) 355-300 nm, IR \( \nu \), vcm\(^{-1} \) 3395 (NH\(_2\)) 1707 (CONH), 2952 (C-H) 1602 (C=C) 7.93-7; 7 (m, 3H o,m dinitroArH), 7.56-7.54 (m, 3HArH), 6.58 (brs, 1H, N=CH), 3.05 (brs2H, NH\(_2\)) confirms synthesis of claimed derivatives. The synthesized compounds were screened further for their antibacterial effect against Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus and Bacillus cereus. All the synthesized derivatives exhibited moderate effect but very good enhancement in the antibacterial potential was exhibited by derivative 7 with nitro group substitution in Schiff’s base showing zone of inhibition as 22, 20, 21, 20 mm followed by compound 6 with 19, 17, 18, 19 mm zone of inhibition well comparable to standard drug Ciprofloxacin and Norfloxacin Norfloxacin 200 µg/disc.

Structure activity relationship studies established the fact that incorporation of nitro group resulted in the enhancement of activity.

**Conclusions**

From the series it can be concluded that compound (7) and (6) showed maximum zone of inhibition against all the strains tested for. So further attempts could be made to extend the series and explore their antibacterial potential to achieve hopeful goal.

**Acknowledgements**

The authors gratefully acknowledge to Director and Management of SBSPGI, Balawala, Dehradun, India for providing the necessary facilities during this experimental study.

Funding: No funding sources
Conflict of interest: None declared

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