

# Microwave assisted synthesis and antimicrobial evaluation of symmetrical 1,2-Phenylenediamine Schiff's base derivatives

## Abstract

Seven Schiff bases were prepared by reacting phenylenediamine with different aromatic aldehyde derivatives. The structures of these compounds were confirmed through different spectroscopic techniques including FT-IR, <sup>1</sup>H-NMR spectroscopy. The synthesised compounds were evaluated *in vitro* for their antimicrobial activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) using agar diffusion method. The antibacterial and antifungal activities of the synthesized compounds were compared with ciprofloxacin and ketoconazole respectively as the standard compounds. Compound **Sw4** showed excellent activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, while compound **Sw3** showed moderate activity against *Candida albicans* only. The obtained results could be considered as a promising and potential antibacterial and antifungal agent; further modification can be performed on the structure of the compound for a improved drug candidate in the future.

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## Introduction

Schiff's bases (imine) are compounds that are containing azomethine group [-HC=N-] in their structure, formed by condensation of an dynamic carbonyl compound with a primary amine.<sup>1,2</sup> Schiff bases have also been shown to show a big range of biological activities, including anti-inflammatory, antibacterial, antifungal, antiproliferative, antimalarial, antiviral, and antipyretic pharmacological activities.<sup>3-7</sup> Azomethine or imine groups can be found in a variety of natural *e.g.* ancistrocladidine (antimalarial), natural-derived *e.g.* chitosan (antifungal), and non-natural compounds *e.g.* N-(Salicylidene)-2-hydroxyaniline (antibacterial). The imine group in such compounds has been shown to be significant to their biological activities.<sup>8-10</sup> Furthermore, the metal complexes of Schiff compounds have been of main attention for a lengthy time due to their capability to join oxygen to redox systems<sup>11</sup> that are oxidizing DNA.<sup>12</sup> They showed noteworthy antimicrobial activity owing to the free radical scavenging capability of their metal composites. Many research groups have been concerned in the chemical synthesis and biological testing of Schiff bases. It has been reported that the synthesized series of 2,4-dichloro-5-fluorophenyl from triazole Schiff bases through aminomethylation with secondary/substituted primary amines and formaldehyde exhibited promising antibacterial and antifungal activity and they found that the aldehyde Schiff base N-aryl thiosemicarbazones had capability as anti-MRSA power and being efficient at nearly half the concentration of the vancomycin.<sup>13</sup>

In addition, Anush et al.,<sup>14</sup> has synthesized a new series of Schiff base by the reaction of Chitosan with different substituted pyrazole-4-carbaldehydes in acidic condition. They also compare Schiff bases and chitosan for their antimicrobial ability against many bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and on fungi such as *Candida albicans*. Their results indicated a higher antibacterial and antifungal inhibitory

effect of these Schiff bases compared to Chitosan and the degree of inhibition diverse with the character of substitution.<sup>14</sup> Dhayabaran and Prakash have synthesized a novel Schiff base, (S,Z)-4-(methylthio)-2-((3-oxo-2,3-dihydro-1H-inden-1-ylidene)amino)butanoic acid (L) and four M(II) complexes (where M = Cu, Co, Zn and Ni) and they found these complexes display superior antioxidant activities against DPPH [2,2-diphenyl-1-picrylhydrazyl] radical and have good *in vitro* antifungal and antibacterial activities.<sup>15</sup> In vision of the extensive attention in the biological activity and outline of Schiff bases derived from aldehydes due to their pharmacological interest, we illustrated here the synthesis and description of seven Schiff bases derived from phenylenediamine with different aromatic aldehyde which, several of them are to the best of our information, have not before been depicted in the literature. The antimicrobial activity of the synthesized compounds was evaluated. MAOS (microwave assisted organic synthesis) has emerged as a innovative lead in organic synthesis. The MAOS technique presents efficient, easy, economic, clean and fast for the synthesis of a huge number of organic molecules. There are many advantages of this technology include extremely accelerated rate of the chemical reaction, improvement in the quality and yield of the product. In addition, the technique is assumed as an significant application in the direction of green chemistry, since this technique is friendly to the environment.<sup>16,17</sup>

## Material and methods

### Chemistry

The entire the chemicals and solvents used in the chemical synthesis of Schiff's base were highest quality and were used without further purification. All the chemical were purchased from Fluka analytical company, Sigma-Aldrich and, UK. Synthesis of compounds was carried out in microwave oven start E which was purchased from Milestone Company, Italy. TLC (Thin layer chromatographic)

analyses were done on pre-coated aluminum plates (silica gel 60778, Fluka analytical). The visualization of TLC spots was performed under UV light. Melting points were determined in open capillary tubes on an Electro thermal SMP30 melting point apparatus (Stuart, UK). The <sup>1</sup>H-NMR spectra were measured on ultra shield Bruker 400 spectrometer using TMS as an internal standard. Finally, the infra-red spectra were measured in Varian FT-IR spectrophotometer 660.

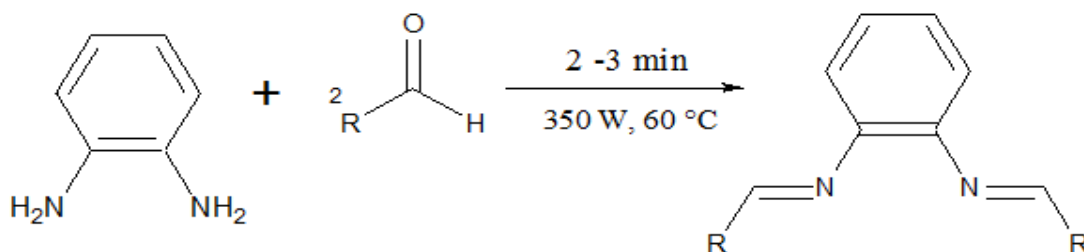
### General procedure for synthesis of Schiff base (Sw1-Sw7)

The Schiff's base was prepared by reaction of one mole of

phenylenediamine and two moles of substituted aromatic aldehydes (Table 1). All reactants were mixed together, and then minimum amount of ethanol was added. This mixture was subjected to microwave irradiation at 350watt for 2-3minutes at 60°C. The development of reaction was watched on TLC. After complete of the reaction, the mixture was left for cooling and then solid product (crude) was gathered by filtration and washed many times with alcohol (ethanol) and then dried utilizing vacuum. The obtained product was redissolved in ethanol for recrystallization and after that dried to give a clean pure product (Scheme 1).

**Table I** Chemical structures of aromatic aldehydes used in chemical synthesis

Compound. No.	R-CHO	Compound. No.	R-CHO
Sw1		Sw5	
Sw2		Sw6	
Sw3		Sw7	
Sw4			



**Scheme 1** General scheme for the synthesis of Schiff base compounds Sw1- Sw7.

## Biology

The antimicrobial activity of all the synthesized compounds (**Sw1-Sw7**) was tested against variety of microorganisms. The microorganisms were purchased from the American type of cell culture collection (ATCC), including *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Candida albicans* (ATCC 10231) at the concentration level of five micromolar by Agar diffusion method.<sup>18</sup> Ketoconazole and ciprofloxacin were used as reference compounds for antifungal and antibacterial activities and they were obtained from Liofilchem, Italy. The antimicrobial results were documented in the form of inhibition zones [diameter, mm] and activity values are shown in Table 3. Minimum inhibitory concentration (MIC) of any compound is defined as the lowest concentration which totally inhibits visible growth (turbidity on liquid media). MIC values were determined by testing performed according to the literature.<sup>18</sup> All determinations were done in triplicate and found the same result.

**Table 2** The physical properties and he spectra data of the synthesized compounds

Comp. NO	M.Wt	M.P (°C)	YILED (%)	FT-IR (cm <sup>-1</sup> ) & <sup>1</sup> H-NMR (ppm)
SW1	344.40	157	95	FT-IR (cm <sup>-1</sup> ): 1609(HC=N), 15605(C=C aromatic), 1361(C-N), 1275(C-O phenolic). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 13(s, 2H, OH), 8.92(s, 2H, CH=N), 7.70-6.95(m, 12H, C-H aromatic).
SW2	372.45	195	93	FT-IR (cm <sup>-1</sup> ): 1609(HC=N), 1509(C=C aromatic), 1353(C-N), 1293(C-O phenolic), 1241(O-CH <sub>3</sub> ). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 13(s, 2H, OH), 8.92(s, 2H, CH=N), 7.70-6.95(m, 12H, C-H aromatic).
SW3	374.52	332	87	FT-IR (cm <sup>-1</sup> ): 1606(HC=N), 1518(C=C aromatic), 1373(C-N). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 8(d, 2H, HC=N), 3.40-3.50(m, 6H, N (CH <sub>3</sub> ) <sub>2</sub> ) 6.88-7.65(m, 12H, Ar-H).
SW4	376.40	208	97	FT-IR (cm <sup>-1</sup> ): 3060(OH), 1611(HC=N), 1580(C=C aromatic), 1362(C-N). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 16.40(s, 2H, OH), 8.88(s, 2H, CH=N), 7.45-6.75(m, 12H, C-H aromatic).
SW5	378.38	234	96	FT-IR (cm <sup>-1</sup> ): 1509(HC=N), 1509(C=C aromatic), 1340(C-N), 1187(N=O). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 8.73(s, 2H, CH=N), 8.4-7.4(m, 12H, C-H aromatic).
SW6	408.49	217	85	FT-IR (cm <sup>-1</sup> ): 1590(HC=N), 1529(C=C aromatic), 1343(C-N). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 5.42(s, 2H, CH=N), 7.70-6.36(m, 10H, C-H aromatic), 3.8-4 (m, 10H, OC <sub>2</sub> H <sub>5</sub> ).
SW7	444.52	226	88	FT-IR (cm <sup>-1</sup> ): 3358(OH), 1617(HC=N), 1569(C=C aromatic), 1317(C-N). <sup>1</sup> H-NMR δ ppm (400MHz, DMSO-d <sub>6</sub> ): 9.7(s, 2H, CH=N), 8.6-7.05(m, 16H, C-H aromatic), 15(S, 2H, OH).

## Biology

The entire synthesized compounds were screened for antimicrobial activity at a concentration 5µM against one Gram (-) strain (*Escherichia coli*), one Gram (+) bacterial strain (*staphylococcus aureus*) and one strain of fungi which is (*candida albican*) by agar diffusion methods. The results obtained from the antimicrobial activity studies of the synthesized Schiff's bases, propose that a few of them have a good activity against the studied microbes. The compound **Sw4** showed excellent activity against *C. albican* compared to ketoconazole, while compound **Sw3** exhibits moderate activity against *C. albican* (Table 3). In addition compound **Sw4** was the only active in the series and was active against *S. aureus* and *E. coli* in addition to the significant activity on *C. albican* (Table 3). The compound **SW3** had a significant activity against *Candida* (15mm) when compared to its activity on

## Result and discussion

### Chemistry

The Schiff's bases (**Sw1-Sw7**) were prepared by condensation of phenylenediamine with aromatic aldehyde at ratio (1:2) and exposure to irradiation of microwave. All the synthesized compounds were purified by successive recrystallization using ethanol, and the purity was checked by performing TLC. A percentage yield of synthetic compounds was excellent. The physical properties of synthesized compounds are described in Table 2. The Infrared spectra of the synthesized compounds confirmed by the existence of stretching of C=N bands at a range of 1500-1690cm<sup>-1</sup> and the nonexistence of carbonyl (C=O) at 1700cm<sup>-1</sup> whereas NH is vanished or hidden underneath the broad band's at 3450-3300cm<sup>-1</sup> in Schiff's base. The <sup>1</sup>H-NMR spectra of the synthesized compounds were recorded in DMSO-d<sub>6</sub>. The chemical shifts (δ), articulated in ppm downfield form TMS (tetramethylsilane) and are shown in Table 2.

bacterial strains irrespectively to its Gram stain. The activity was due to dimethyl amine moiety at position *para* to the aldehyde indicates evidence of selectivity of the ligand to the target in *Candida*.

The **Sw4** structure-activity relationship potency against *S. aureus*, *E. coli* and *C. albicans* could depend to a large extent on their ability to interact with thiol and hydroxyl of the benzene.<sup>19</sup> Furthermore, the location of the hydroxyl group is powerfully involved, the *ortho*-position and not the *para*- or the *meta*-being the most constructive, because of a mechanism by which the *o*-hydroxyl groups are concerned in intermolecular hydrogen bond configuration assisting the addition of thiols and these results are consistent with the literature where Dinkova et al.,<sup>20</sup> found these *ortho*-hydroxyl groups exhibit a tendency for huge intermolecular hydrogen bond formation, which may elevate the reactivity and help addition of mercaptans, thus

increasing inducer potencies. Finally, further study should be done on the mode of action of these compounds to explicate the structure to function correlation. According to different studies published on some Schiff base compounds, it was suggested that the mode of action may engage a variety of targets in microorganisms.<sup>21-23</sup> These mechanisms could be classified into four points:

- a. Inhibition of various cellular enzymes which are significant in the microorganism's metabolic pathway
- b. The intrusion with the cell wall synthesis as a result the cell permeability which may be changed or they may muddle the lipoproteins leading to cell deaths,

- c. Destroying of one or more proteins of the cell, as a result of which the normal cellular processes are harmed.
- d. Configuration of a hydrogen bond through the azomethine group with the active centres of cell components resulting in a conflict with the ordinary cell processes.

Due to the relationship in chemical structures with the previously studied compounds by many groups we could hypothesize that one of the earlier suggested targets could be well thought-out for our compounds. But our postulation should be proved by additional biochemical studies in the future.

**Table 3** Zone of inhibition (mm) and the MIC between brackets ( $\mu\text{g/ml}$ ) data of the synthesized compounds

Compounds	Antibacterial activity		Antifungal activity
	<i>S. aureus</i> <sup>a</sup>	<i>E. coli</i> <sup>a</sup>	<i>C. albicans</i> <sup>a</sup>
SW1	-	-	-
SW2	-	-	-
SW3	-	-	15±0.96 (0.09)
SW4	29±0.35 (0.32)	25±0.92 (0.26)	25±1.10 (0.12)
SW5	-	-	-
SW6	-	-	-
SW7	-	-	-
Ciprofloxacin	27±0.32 (0.30)	33±0.46 (0.28)	-
ketoconazole	-	-	23±0.82 (0.13)

The dash (-) indicates an absence of activity, <sup>a</sup> Zone of inhibition (mm) data are presented as mean values  $\pm$  standard deviation calculated from triplicate determinations. MIC values are given in brackets ( $\mu\text{g/mL}$ ). Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit bacterial growth.

## Conclusion

In conclusion, seven Schiff bases were synthesized by reacting phenylenediamine with different aromatic aldehyde derivatives. The structures of the synthesised compounds were confirmed by diverse spectroscopic methods. The synthesized compounds were also biologically evaluated and they were tested against bacteria: *S. aureus*, *E. coli* and fungi: *C. albicans*. Compound **SW4** exhibited good growth inhibition activity against bacteria and *Candida* and it is promising to act as a possible antimicrobial agent. Results also exposed that compound **SW3** showed moderate activity against *Candida*; additional structural alterations might lead to a competent anticandida agent which could be used as anticandida drugs, particularly if additional work established that cytotoxicity of the synthesized Schiff bases is less than Nystatin.

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None.

## Conflict of interest

The author declares that there is no conflict of interest.

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