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A facile and efficient synthesis of some odorant Schiff bases using microwave irradiation and their antimicrobial activity

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Three odorant Schiff bases of methylanthranilate, an aroma constituent of blossom essential oils, with naturally occurring odorant aldehydes- anisaldehyde, benzaldehyde and cinnamaldehyde employing microwave (MW) irradiation method were synthesized. Elemental and spectral (FT-IR, ^{1}H-NMR) analysis of these compounds supported their molecular structures. Olfactory properties of these Schiff bases indicated their suitability for use in various fragrance compositions. These compounds also showed varied antimicrobial activity against *Aspergillus niger*, *Penicillium chrysogenum*, *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Schiff base, methylanthranilate, aldehydes, microwave irradiation, olfactory property, antimicrobial activity

INTRODUCTION

Schiff bases (azomethines / anils / imines) having azomethine group (-CH=N-) belong to a group of compounds which possess varied biological activities including antimicrobial, anticancer, antineoplastic, antiviral, antitumour, anti-HIV, antitubercular, anticonvulsant, anthelmintic, antiplatelet, antimalarial, antibiotics, anti-inflammatory, diuretic, and analgesic [1-18]. Besides, Schiff bases are also well known in flavoring and perfumery due to their odor characteristics [19]. Several Schiff bases are reported to exhibit floral, fruity odors, namely of the citrus type and, more particularly, reminiscent of the odor of the orange-flower [20-21]. Schiff bases also act as intermediates for producing other fragrance materials [22-23]. Condensation of carbonyl compounds (aldehydes or ketones) with primary amines yields Schiff bases with biological and olfactory properties. Microwave (MW) irradiation technique being simple, clean, fast, efficient and economical is in tune with the green chemistry approach and therefore MW assisted synthesis of organic compounds has acquired significant importance over conventional organic synthesis in terms of reduced solvent consumption and reaction time, improved yields and easier workup [24-28]. Several Schiff bases have been synthesized using MW irradiation and characterized [29-34].

Present paper describes synthesis of three odorant Schiff bases of methylanthranilate (Figure **1**), an aroma constituent of blossom essential oils of neroli, ylang-ylang, and citrus [35], with naturally occurring odorant aldehydes - anisaldehyde, benzaldehyde and cinnamaldehyde) (Figure **2**) using MW irradiation method. The structures of the synthesized compounds were confirmed by means of their physical (elemental analysis) and spectral (FT-IR and ^{1}H-NMR) data. Their olfactory and antimicrobial properties were also evaluated.

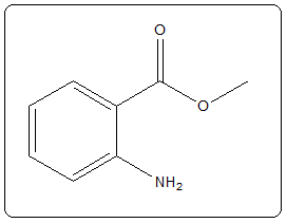

Figure 1: Methylanthranilate

Synthesis of these Schiff bases using conventional method and their odoriferous properties are reported in

literature [36]. To the best of our knowledge synthesis of these Schiff bases under influence of MW irradiation is being reported for the first time. This is also first report on antimicrobial activity of the synthesized Schiff bases.

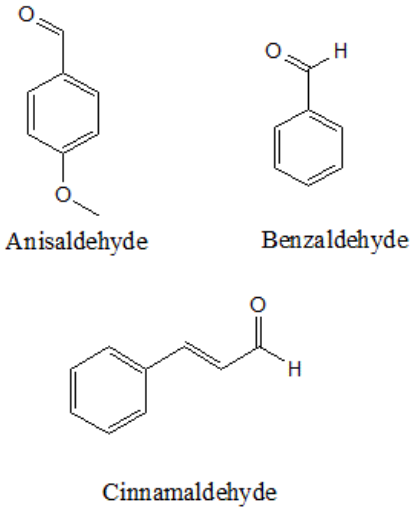

Figure 2: Reactant aldehydes

MATERIALS AND METHODS

General

All chemicals obtained from commercial suppliers were of highest purity grade and used without further purification. These include anisaldehyde (Loba chemie), benzaldehyde (Merck), cinnamaldehyde (Himedia), methylanthranilate (Himedia), Nutrient Agar Media (Himedia) and Potato Dextrose Agar (Himedia). Elemental analysis of carbon, nitrogen and hydrogen were carried out on CHN elemental analyser (Thermo Scientific). Infrared (IR) spectra were recorded at room temperature from 4000 cm^{-1} to 500 cm^{-1} with KBr pellets at a resolution of 4cm^{-1}, using Thermo-Nicolet 8700, Research spectrometer (Thermo Scientific). ^{1}H-NMR spectra were recorded on Bruker Avance-II 500 MHz instrument using $CDCl_3$ as solvent and TMS as an internal slandered. Chemical shifts (δ) are reported in ppm. The microwave assisted synthesis was performed in scientific microwave oven, (SRL-Milestone Start S Labstation for synthesis, (Operating between 140-1600W).

General procedure for preparation of Schiff bases

Solvent free MW irradiation method: The equi-molar ratio of methylanthranilate (0.1mol, 15.11 g) and aldehyde (0.1mol: anisaldehyde, 13.61 g; benzaldehyde, 10.61g; cinnamladehyde, 13.21g) were mixed in a reaction flask and irradiated with MW at 300 for 4min. After completion of the reaction, the mixture was purified by vacuum distillation. These products were stored at 4ºC until analysed further.

Methyl- 2-(4-methoxybenzylideneamino)benzoate (Sb1)

$C_{16}H_{15}NO_3$; Intense yellow; Yield: 69% (MW irradiation method); Fraction condition: 153-155°C/0.0079MPa; IR (KBr, cm^{-1}): 1727, 1621, 1492, 1236, 1140; ^{1}H-NMR (500 MHz, $CDCl_3$, (δ) ppm): 8.45 (1H, s, HC=N), 6.66-7.91 (8H, m, Ar-H), 3.91 (3H, s, $OCOCH_3$), 3.89 (3H, s, OCH_3); Anal. Calc. for $C_{16}H_{15}NO_3$: (%) calculated: 71.34, 5.61, 5.20, 17.83, found: 71.39, 5.67, 5.18, 17.76; MW: 269.10

Methyl 2-(benzylideneamino)benzoate (Sb2)

$C_{15}H_{13}NO_2$; intense yellow; Yield: 79% (MW irradiation method); Fraction condition: 130-132°C/0.0079MPa; IR (KBr, cm^{-1}): 1714, 1610, 1504, 1253, 1160; ^{1}H-NMR (500 MHz, $CDCl_3$, (δ) ppm): 8.49 (1H, s, HC=N), 6.92-7.89 (9H, m, Ar-H), 3.89 (3H, s, $OCOCH_3$); Anal. Calc. for $C_{15}H_{13}NO_2$ (%) calculated: 75.28, 5.47, 5.85, 13.37, found: 75.33, 5.46, 5.84, 13.46; MW: 239.09

Methyl 2-(3-phenylallylideneamino)benzoate (Sb3)

$C_{17}H_{15}NO_2$; reddish brown; Yield: 69% (MW irradiation method); Fraction condition: 153-155°C/0.0079MPa; IR (KBr, cm^{-1}): 1717, 1613, 1488, 1228, 1139; ^{1}H-NMR (500 MHz, $CDCl_3$, (δ) ppm): 7.84 (1H, s, HC=N), 6.61-7.36 (9H, m, Ar-H), 6.61 (1H, s, vinylic H), 5.79 (1H, s, vinylic H), 3.82 (3H, s, $OCOCH_3$); Anal. Calc. for $C_{17}H_{15}NO_2$ (%) calculated: 76.94, 5.70, 5.28, 12.06, found: 76.93, 5.79, 5.31, 11.98; MW: 265.11

Olfactory evaluation:

Olfactory properties of the Schiff bases were studied employing standard olfactory method [37]. Sample solutions (10 wt. % in ethanol) were used to evaluate their odour properties.

Antimicrobial Properties:

Disc diffusion method

Biological studies of the Schiff bases was evaluated according to the guidelines of the National Committee for Clinical Laboratory Standards [38] using the agar disc diffusion method. The bacterial cultures for *Staphylococcus aureus* (ATCC 259235) and *Escherichia coli (*ATCC 27853) were obtained from Microbial Type

Culture Collection (MTCC), Chandigarh and fungal cultures of *Aspergillus niger* and *Penicillium chrysogenum* were obtained from National Type Culture Collection (NTCC), Forest Research Institute, Dehradun. Exponentially grown cultures of bacteria and fungi were mixed with sterile 0.85% saline solution to make the final volume upto 10^5- 10^6 cfu/ml. Petri plates containing 15 ml of Nutrient Agar Media (NAM) and Potato Dextrose Agar (PDA) were used for bacteria and fungi respectively. The inoculum of bacterial and fungal cultures was spread separately on the surface of their respective solidified media. Schiff bases (40 µg/ml) were dissolved in dimethylsulfoxide (DMSO) and whatman no.-1 filter paper discs (5mm in diameter) impregnated with the test compound (5 µl/disc) were placed on the plates. Ciprofloxacin (10µg/disc) was used for bacteria and griseofulvin was used for the fungi as positive control. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24h at 30±2 ºC and the fungal cultures were incubated for 5-6 days at 27±2ºC. The inhibition zone diameters were measured in millimeters. All the tests were per formed in triplicate and the average was taken as final reading.

Determination of MIC

MIC values were determined by testing performed according to the guidelines of NCCLS [39]. Solutions of the test compounds, and control compounds (antibacterial – ciprofloxacin and antifungal- griseofulvin) were prepared in DMSO at different concentrations of 10, 20, 30, 40, 50, 60 µg/ml to determine the MIC. All determinations were done in triplicate and found the same result.

RESULTS AND DISCUSSION

Three odorant Schiff bases namely methyl- 2-(4-methoxy benzylideneamino) benzoate (Sb1), methyl 2-(benzylideneamino)benzoate (Sb2), methyl 2-(3-phenyl allylidene amino)benzoate (Sb3) (Figure **3**) were prepared from naturally occurring three aldehydes anisaldehyde, benzaldehyde, cinnamaldehyde, respectively with methylanthranilate by microwave (MW) irradiation method. A large number of experiments were performed to optimize the irradiation power and its duration and the optimized conditions are reported. MW irradiation afforded no solvent consumption, fast reaction (occurred in 4 minutes) with 69%, 79% and 69%yields, respectively.

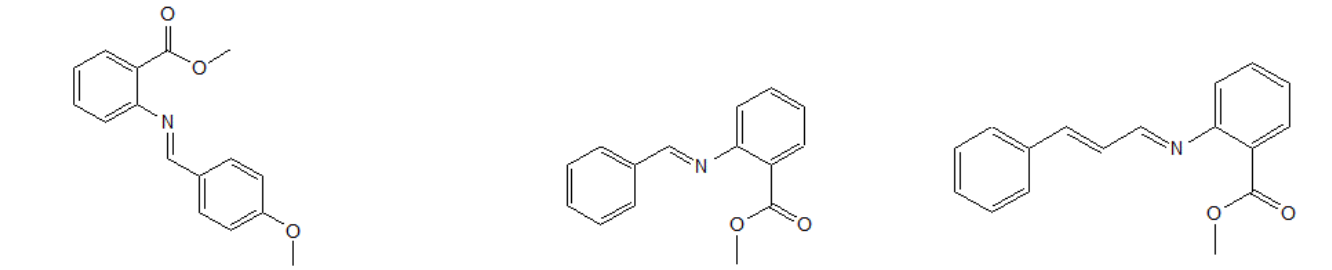

Figure 3: Synthesized odorant Schiff bases

The Schiff bases were characterized by analysis of the elements (C, H and N), FT-IR and ^{1}H-NMR spectral data. Elemental analysis of the Schiff bases was in agreement to their molecular formula assigned. The FT-IR spectra of the free aldehydes (anisaldehyde, cinnamaldehyde and cuminaldehyde) have a strong band at 1690-1700 cm^{-1} (due to carbonyl stretch), two bands of moderate intensity in the region 2700-2800 cm^{-1} (due to H-C=O stretch), while two sharp bands at 3500 and 3580 cm^{-1} corresponding to N-H stretch were observed in the IR spectrum of methylanthranilate. In the IR spectra of the Schiff bases, these bands disappeared and a new band appeared in 1621, 1610 and 1613 cm-1 attributed to the v(C=N) mode of azomethine linkage showing the condensation between the -CHO group of the aldehydes and amino group of methylanthranilate resulting in the formation of the respective Schiff bases.

The ^{1}H-NMR spectra of the odorant Schiff bases exhibit singlet of integration intensity equivalent to one hydrogen at 7.84-8.49 ppm and attributed to (-CH=N-). A singlet at 3.82-3.91 ppm due to ester protons was observed in the spectra of all the Schiff bases. A broad signal at 5.74 ppm due to free NH_2 protons in the spectra

of methylanthranilate was absent in the observed spectra of the Schiff bases which indicated the formation of the azomethine linkage. The multiplets within the 6.61-7.91 ppm range were assigned to protons belonging to aromatic rings. The spectra of Sb1 showed a singlet with an integration equivalent to three hydrogens at 3.89 ppm corresponding to the methoxyl protons. The NMR spectral assignments of the Schiff bases support the inferences drawn from the elemental and IR studies.

Olfactory evaluation

Schiff bases were evaluated for their olfactory properties in terms of their odor profile and odor strength and data are shown in Table **1**. The Schiff bases possessed an interesting odor profile having contributions of the characteristic aroma of methyanthranilate and respective aldehydes which differed in the notes with medium strength. Sb1 and Sb2 had floral, fruity undertone in their odor profile with strong sweet, bitter almond notes, while Sb3 showed spicy, sweet, cinnamon like odor due to their reactant aldehydes. The olfactory properties of these Schiff bases indicated their possible use in varied fragrance and flavour compositions.

Table 1: Olfactory properties of the Schiff bases

Sl. No.	Schiff base	Odor profile	Odor strength
1.	Sb1	Strong sweet floral with fruity undertone	Medium
2.	Sb2	Bitter almond with mild floral fruity tone	Medium
3.	Sb3	Spicy, sweet, cinnamon like	Medium

Antimicrobial activity

Antimicrobial activity of Sb1, Sb2 and Sb3 was examined against four pathogenic microorganisms which include one gram positive bacteria (*Staphylococcus aureus*), one gram negative bacteria (*Escherichia coli*) and two fungi viz; *Aspergillus niger* and *Penicillium chrysogenum* All the Schiff bases showed moderate to good activity against the pathogenic organisms tested. The solvent used for the preparation of compound solutions (DMSO) did not show inhibition against the tested organisms (negative control). Compared to the positive control, compounds showing the zones of inhibition between 9-16 mm and >17 mm were considered to be moderately active and highly active, respectively. Zone of inhibition, MIC and % activity index of the tested compounds are given in Table **2**.

The MIC values of the highly active compounds ranged between 20-30 µg/ml with 66.66-75.00% activity index while those for moderately active compounds were 40µg/ml with 43.75-53.70% activity index. Compounds Sb3 found highly active while compounds Sb1 and Sb2 found moderate active against all four microorganisms tested.

Table 2: Zone of inhibition, MIC and % activity index of odorant Schiff bases

Schiff base	Bacteria Zone of inhibition (MIC)		Fungi Zone of inhibition (MIC)		*% Activity index (Bacteria)		% Activity index (Fungi)	
	E. coli	***S. aureus***	***A. niger***	***P. chrysogenum***	***E. coli***	***S. aureus***	***A. niger***	***P. chrysogenum***
Sb1	13.0 (40)	14.5 (40)	11.5 (40)	10.5 (40)	52	53.70	47.91	43.75
Sb2	13.0 (40)	12.5 (40)	10.0 (40)	11.00 (40)	52	48	46.29	45.83
Sb3	18.5 (20)	18.0 (20)	18.0 (20)	17.5 (20)	74	66.66	75.00	72.91
Ciprofloxacin	25.0 (20)	27.0 (20)	-	-	-	-	-	-
Griseofulvin	-	-	24.0 (20)	24.0 (20)	-	-	-	-
DMSO	-	-	-	-	-	-	-	-

1 - 8 mm = less active; 9 - 16 mm = moderate active; >17 mm = highly active

***% Activity index** = Zone of inhibition by test compound (diameter) / Zone of inhibition by control compound (diameter) x 100

CONCLUSION

MW irradiation technique provided a facile, rapid, clean and effective synthesis of three Schiff bases namely methyl-2-(4-methoxy benzylideneamino) benzoate, methyl 2-(benzylideneamino)benzoate, and methyl 2-(3-phenyl allylidene amino) benzoate. Olfactory attributes of these Schiff bases were indicative of their use in various fragrance compositions. These compounds also displayed moderate to good antimicrobial activity against bacteria - *Staphylococcus aureus* and *Escherichia coli*, and fungi- *Aspergillus niger* and *Penicillium chrysogenum.*

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