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Bioefficacy evaluation of novel N-alkyl-2-hydroxyketimines (schiff bases)

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ABSTRACT

Twelve new *ortho*-Hydroxyketimines were synthesized during 2006–07 by conventional as well as microwave method and evaluated for their antifeedant activity against the lepidopteran insect pests, *Acheae janata* (L.) and *Spodoptera litura* (L.). Conventional methods for synthesis of Schiff bases require refluxing at 140° C of the reactants in different solvents for at least 24 hr or more, whereas the microwave-assisted synthesis has brought down the reaction time from 24 hr to 1 min. The procedure does not require any organic solvents and the time has been reduced to only 1 min. Comparative yields of all compounds by different methods revealed that the yield was low in conventional method (79–87%) as compared to microwave-assisted synthesis (94–97%). The bioassay revealed that all the test compounds exhibited promising insect antifeedant activity; N-hexyl-2-hydroxyacetophenonimine being the most effective with antifeedant index value of 100, followed by N-hexyl-2-hydroxypropiophenonimine with antifeedant index value of 81.32 against *A. janata* and antifeedant index value of 100 of N-hexyl-2-hydroxypropiophenonimine against *S. litura*. The results obtained from bioassay indicated that this class of compounds has not only given a lead with regard to potential of Schiff bases in pest control, but has suggested that a carbon chain length of 6 atoms in the atoms in the side chain is optimum on the basis of structure activity relationship (SAR).

Key words: *Achaea janata*, Antifeedant, Environmentally benign, Microwave, Schiff's bases, *Spodoptera litura*

The synthesis of imines is of biological importance as they are reported to possess various activities such as, antifungal (Manrao *et al*. 2005), nitrification inhibitor (Datta *et al*. 2001), nematicidal (Manrao and Kaul 1999) and also important precursors for the synthesis of several biologically versatile heterocyclic rings, e.g., 1, 3-benzoxazines, pyrazoles and dithiazolidinones (Shakil *et al*. 2005) but could not be exploited to their potential in pest control due to high temperature (150°C) and long period of time (12–24 hr) required for completion of the reaction (Shakil *et al*. 2003, Tumerdem *et al*. 2005, Regina *et al*. 2004, Wen and Zhuo 2003) and Mart *et al*. 2004), thus reactants become hazardous. Microwave-assisted synthesis of imines not only minimizes the reaction time but also reduces the risk of exposure. It has several advantages over conventional technology: remarkable decrease in the time necessary to carry out reactions; avoid heating to high temperature and without the use of volatile solvents; improved isolated yields of products; and sometimes remarkable effects on chemo-, regio- and stereo- selectivity. Microwaves have a specific 'microwave effect' that lowers the activation energy of a reaction. Thus, microwave - mediated organic reactions take place more rapidly, safely and with high yields. Moreover, the microwave - assisted organic synthesis (MAOS) or microwave-induced organic reaction enhancement (MORE) chemistry techniques constitute an emerging technology making industrially important organic syntheses more eco-friendly than conventional reactions. Environmentally benign character of the organic synthesis is desirable for large-scale industrial application of Schiff bases owing to their versatile biological properties.

The castor semi-looper, *Achaea janata* (Linnaeus) is a noctuid moth feeding on castor, *Ricinus communis* L. plants. As they feed voraciously on the castor oil plant causing severe losses to the seed yield, the control of these pests is essential. Loopers can cause considerable defoliation of lychees and longans also in all areas (Jyothi 2005).

Spodoptera litura (Fab.) is major polyphagus pest, distributed throughout south and eastern world infesting 112 species of plants belonging to 44 families, of which 40 species

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are known from India (Chari and Patel 1972). In India *S. litura* has been reported as an increasingly important pest during the rainy (*kharif*) seasons causing heavy yield loss throughout India (Amin 1983).

Our group has reported the synthesis and antinemic properties of Schiff bases (Kundu *et al.* 2008). In continuation of our work, we have synthesized 12 substituted Schiff bases by MORE technique and compared the progress and yield of products with that of conventional method. All the Schiff bases were tested against *A. janata* and *S. litura*.

MATERIALS AND METHODS

Melting points were determined by using open capillaries in sulphuric acid bath and are given uncorrected. Thin layer chromatography was performed on 200ìm thick aluminium sheets having silica gel 60 F_{254} as adsorbent. The solvent system used for developing the TLC plate was petroleum ether : chloroform (9:1 by volume). Spots were visualized under UV-light. The IR spectra were recorded on either a Perkin-Elmer 2000 FT-IR or RXI FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 Avance spectrometer at 300 and 75.5 MHz, respectively using tetramethylsilane (TMS) as an internal standard. The elemental analysis was done on a Eurovector Elemental analyzer 3000 using sulphanilamide as standard with linear calibration. Laboratory grade reagents and solvents were locally procured.

In the conventional process equimolar amounts of different chain length of amines (0.025 mol), *o*-hydroxy-acetophenone (0.025 mol, 3.4 g) or *o*-hydroxypropiophenone (0.025 mol, 3.75 g) and xylene (20 ml) were mixed together in a 150 ml round-bottom flask and fitted with a reflux condenser. The mixture was refluxed for 72 hr in oil bath at 140–145°C. Xylene was evaporated in a rotary evaporator at 75–85°C. The compound was cooled to room temperature to let the product to solidify and bright yellow solid separated.

In microwave - assisted synthesis equimolar amount of *o*-hydroxyacetophenone (0.068 g, 0.0005 mol) or *o*-hydroxy propiophenone (0.075 g, 0.0005 mol) and different alkyl amines were mixed in a 50 ml beaker. The reaction mixture was irradiated inside a microwave oven along with distilled water as dummy and the progress of reaction was monitored periodically by TLC in petroleum ether: chloroform (9 : 1 by volume) solvent system. The reaction was complete within 1 min. The reaction mixture was cooled to room temperature, and yellow solid separated, which on recrystallization with petroleum ether: chloroform (9 : 1 by volume) mixture gave bright yellow crystals having sharp melting point.

The spectroscopic data of representative compound is given as follows:

N-propyl-2-hydroxypropiophenonimine (1): It was obtained as a bright yellow solid (0.0905g) in 94.76% yield, R_f : 0.47 (petroleum ether:chloroform, 9:1); IR (nujol)/cm: 3145 (OH), 2906 (C-H), 1620 (C=N), 1600, 1590, 1500 and 1460 (aromatic ring), 1455, 1065 and 832. ^{1}H (300 MHz, $CDCl_3$) : □ 1.06 (t, 3H, *J* = 6.0 Hz, H-3"), 1.65-1.98 (q, 2H, H-2"), 2.26 (t, 3H, H-2'), 2.83 (m, 2H, H-1'), 3.62 (t, 2H, *J* = 6.2 Hz, H-1"), 6.65-6.72 (m, 1H, H-5), 6.85 (dd, 1H, *J* = 8.0 and 0.9 Hz, H-3), 7.18-7.30 (m, 1H, H-4), 7.52 (dd, 1H, *J* = 8.0 and 0.9 Hz, H-6),16.67 (br s,1H, chelated OH).

The data for other Schiff bases are same with only difference in side chain signals.

N-hexyl-2-hydroxyacetophenonimine (2): It was obtained as a bright yellow solid (0.1055 g) in 96.35% yield, R_f : 0.46 (petroleum ether:chloroform, 9:1).

N-hexyl-2-hydroxypropiophenonimine (3): It was obtained as a bright yellow solid (0.1115 g) in 95.71% yield, R_f : 0.48 (petroleum ether:chloroform, 9:1).

N-heptyl-2-hydroxyacetophenonimine (4): It was obtained as a bright yellow solid (0.1090 g) in 93.56% yield, R_f : 0.46 (petroleum ether:chloroform, 9:1).

N-heptyl-2-hydroxypropiophenonimine (5): It was obtained as a bright yellow solid (0.1198 g) in 97.0% yield, R_f : 0.45 (petroleum ether:chloroform, 9:1).

N-nonyl-2-hydroxypropiophenonimine (6): It was obtained as a bright yellow solid (0.1323 g) in 96.21% yield, R_f : 0.47 (petroleum ether:chloroform, 9:1).

N-undecyl-2-hydroxypropiophenonimine (7): It was obtained as a bright yellow solid (0.1495 g) in 98.68% yield, R_f : 0.45 (petroleum ether:chloroform, 9:1).

N-pentadecyl-2-hydroxyacetophenonimine (8): It was obtained as a bright yellow solid (0.1685 g) in 97.68% yield, R_f : 0.45 (petroleum ether:chloroform, 9:1).

N-hexadecyl-2-hydroxyacetophenonimine (9): It was obtained as a bright yellow solid (0.1745 g) in 97.22% yield, R_f : 0.49 (petroleum ether:chloroform, 9:1).

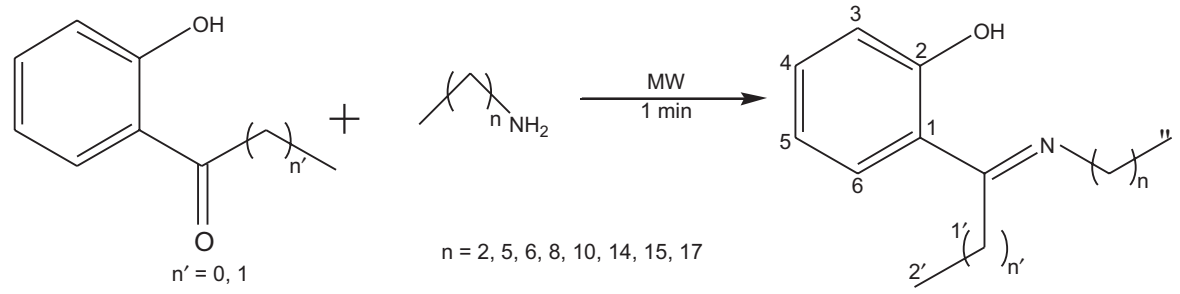
N-hexadecyl-2-hydroxypropiophenonimine (10): It was obtained as a bright yellow solid (0.1802 g) in 96.62% yield, R_f : 0.46 (petroleum ether:chloroform, 9:1).

N-octadecyl-2-hydroxyacetophenonimine (11): It was obtained as a yellow solid in 94.68% yield, R_f : 0.49 (petroleum ether:chloroform, 9:1).

N-octadecyl-2-hydroxypropiophenonimine (12): It was obtained as a yellow solid in 96.71% yield, R_f : 0.47 (petroleum ether:chloroform, 9:1).

S. litura Fab. and *A. janata* L. larvae used in this study were obtained from a laboratory colony of Department of Entomology, Directorate of Oilseed Research, Rajendra Nagar, Hyderabad. The culture has been continuously maintained on castor bean leaves (*R. communis* L.) at room temperature (25 ± 2ºC), 65 ± 5% RH and 16:8 L:D photo period in the laboratory, Indian Institute of Chemical Technology, Hyderabad.

Antifeedant assay: Leaf disc bioassays were carried out to determine antifeedant activity of Schiff bases. The third instar larvae of *S. litura* and *A. janata* were starved for 3 hr. Leaf discs (21 cm²) were punched from castor bean leaves, and sprayed separately with different dosages of 50, 75 and



Scheme 1

100 mg of compounds in 100 µl of acetone. The treated discs were air-dried for about 3 min. to evaporate the solvent. Control discs were sprayed with solvent acetone. A choice test was performed in a 14 cm dia. Perti-dish lined with moistened Whatman (No.1) filter paper. In choice test, the area was divided in equal quadrants, each quadrant containing a treated or control disc placed alternately. Two third-instar larvae of each like *S. litura* and *A. janata* were placed separately in the center of the dish. There were 10 replicates for each treatment and all the treatments were repeated on 3 different days. The percentage antifeedant index was calculated from the following formula (Abbott 1925):

$$\text{Antifeedant index} = [(C - T) / (C + T)] \times 100$$

where C is the weight of leaf disc consumed in the control and T is the weight of leaf discs consumed in the treatment.

RESULTS AND DISCUSSION

Synthesis of Imines

The test compounds were prepared by condensation of *o*-hydroxyacetophenone or *o*-hydroxypropiophenone with different amines of varying chain lengths, both by conventional method and by MORE technique in quantitative yields (Scheme 1).

Comparative yields of all compounds by different methods, as shown in Table 1, revealed that the yield was low in conventional method (79-87%) as compared to microwave - assisted synthesis (94-97%) of imines. The time required was only 1 min. when the compounds were synthesized by MORE technique compared to up to 72 hr of heating required in conventional method.

Absorption at 1620 / cm for C=N moiety in IR spectra and at 3.62–3.94 as triplet for 2 protons with J = 6.2 of –N-CH_2- group of side chain in NMR spectrum of all the products (1-12) confirmed the condensation of carbonyl group with amino group of long chain amines. With acetophenones as the carbonyl compound, a common signal appeared invariably at 2.26–2.46 (3H, s); whereas in case of propiophenones, in addition to the signal, another quartet at 2.76–2.98 for C-CH_2, integrating for two protons was observed. The compounds showed signals at aromatic region placed at ä 6.35-6.75 (5H, m), 6.82–6.90 (1H, dd, J = 8.0 and 0.9 Hz), at 7.18–7.30 (1H, m) and another at 7.52–7.59 (1H, dd, J = 8.0 and 0.9 Hz). Another common signal appearing at 16.56–16.67 indicated the chelation between nitrogen of –C=N- and hydrogen of 2-OH group in all the products. In addition to that a triplet at 1.01 integrating for 3 protons (J = 6.0) of methyl group and a multiplet from 1.05–1.38 integrating for 32 protons for methylene groups confirmed the formation of N-octadecyl-2-hydroxy-acetophenonimine (1). The spectral features of other imines

Table 1 Structure, physical and analytical data for the substituted Schiff bases

Compound	R	n	mp (ºC)	Yield (%)		Calculated (%)			Observed (%)		
				Conventional method	Microwave method	C	H	N	C	H	N
1	C_2H_5	2	73	83.61	94.76	75.39	8.90	7.32	75.21	8.83	7.57
2	CH_3	5	72	80.12	96.35	76.71	9.59	6.39	76.39	10.01	6.32
3	C_2H_5	5	73	78.18	95.71	77.25	9.87	6.01	77.42	9.98	5.67
4	CH_3	6	75	80.38	93.56	77.25	9.87	6.01	77.16	9.92	5.97
5	C_2H_5	6	74	80.80	97.00	77.73	10.12	5.67	77.85	10.06	5.77
6	C_2H_5	8	70	79.12	96.21	78.67	10.55	5.09	78.67	10.38	5.15
7	C_2H_5	10	72	82.51	98.68	79.21	10.89	4.62	79.00	11.02	4.65
8	CH_3	14	69	81.27	97.68	80.00	11.30	4.06	79.90	11.42	4.11
9	CH_3	15	74	78.88	97.22	80.22	11.42	3.90	80.40	11.31	3.90
10	C_2H_5	15	71	81.39	96.62	80.43	11.53	3.75	80.39	11.43	3.86
11	CH_3	17	76	83.61	94.68	80.62	11.63	3.62	80.87	11.45	3.49
12	C_2H_5	17	74	77.90	96.71	80.80	11.72	3.49	80.91	11.62	3.67

Table 2 Antifeedant Index of substituted Schiff bases against *Achaea janata* and *Spodoptera litura* by leaf disc method

Compound*	Antifeedant index**	
	A. janata	*S. litura*
1	51.40	98.63
2	100.0	60.69
3	81.32	100.00
4	67.65	0.00
5	14.67	98.91
6	17.49	99.08
7	26.13	99.64
8	0.00	0.00
9	0.00	0.00
10	4.59	0.00
11	19.54	0.00
12	0.00	0.00

*Tested at 10mg/21cm^2 ; **Antifeedant index [(C-T)/(C+T)] × 100, where C and T represent the amount of leaf eaten of control and treated leaf disc respectively; Standard azadirachtin A active at 2mg/21cm^2

were similar except side chain length.

Although all the compounds possessed activity against both *A. janata* and *S. litura* it was observed that the antifeedant index increases with the number of carbon atoms in the side chain of the molecule and reaches a maximum at carbon chain length of 6 and then starts decreasing, i e increases from C 3 to C 6 (against *A. janata* (Table 2). Compound 1, having 3 carbon atoms in the side chain, has antifeedant index 51.40, 2, having 6 carbon atoms in the side chain has antifeedant index 100%. The activity decreases after that and 4, having carbon atom 7, was found to have antifeedant index 67.65 and so on. In case of *S. litura*, the observation was more or less the same as is evident from Table 2. Thus it was observed that bioactivity increases with increase in chain length upto 6 carbon atoms whereas further increase in chain length decreases the activity. However, the commonly used chemical azadirachtin A has 100% antifeedant index at 2mg/cm^2.

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