Med Chem Res (2013) 22:4360–4366
DOI 10.1007/s00044-012-0450-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of fatty acid Schiff base esters as potential antimicrobial and chemotherapeutic agents

Y. Mohini · R. B. N. Prasad · M. S. L. Karuna · C. Ganesh Kumar · M. Poornima · P. Sujitha

Received: 1 September 2012 / Accepted: 21 December 2012 / Published online: 8 January 2013

Abstract Schiff base {4-[(pyridine-3-ylmethylimino)-methyl] phenol} was prepared by reacting 4-hydroxybenzaldehyde with 3-aminomethylpyridine in ethanol for 6 h (85 % yield) followed by their esterification via reaction with fatty acids of varying chain lengths. The structure of these Schiff base esters were elucidated using chromatographic and spectroscopic techniques like GC–MS, ^{1}H NMR, ^{13}C NMR, and ESI–MS. Schiff base esters with shorter chain heptanoic (**2a**) and decanoic acid (**2c**) showed good activity against all the tested bacterial and fungal strains. The synthesized esters were also studied for cytotoxicity toward different human tumor cell lines like HeLa, HepG2, A549, MDA-MB-231, and MCF 7 and Neuro2a; however, Schiff base esters with shorter chain (**2a**, **2b**) and medium chain fatty acids (**2d**, **2i**) exhibited good anticancer activity and selectively toward MDA-MB-231 and MCF 7, while long chain fatty acid (**2g**) Schiff base ester exhibited good anticancer activity selectively toward MDA-MB-231 as compared to the parent molecule, Schiff base (**1**).

Keywords Schiff base esters · Vegetable oil based Schiff base esters · Antimicrobial agents · Anticancer agents

Electronic supplementary material The online version of this article (doi:10.1007/s00044-012-0450-y) contains supplementary material, which is available to authorized users.

Y. Mohini · R. B. N. Prasad · M. S. L. Karuna (✉)
Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500 607, India
e-mail: karuna@iict.res.in

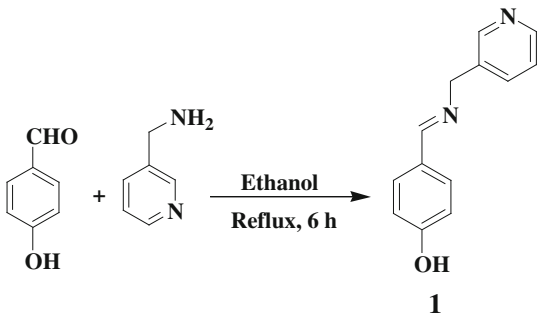
C. Ganesh Kumar · M. Poornima · P. Sujitha
Chemical Biology Laboratory, CSIR-Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500 607, India

Introduction

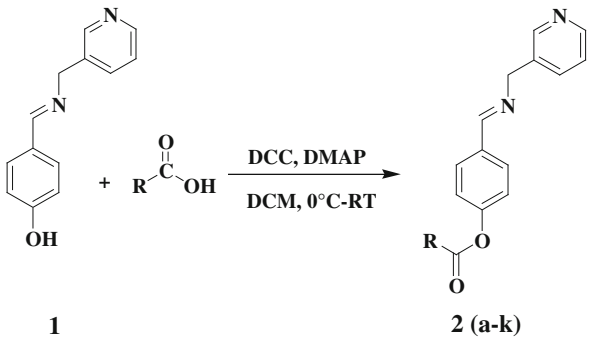
Compounds containing azomethine CH=N structure referred to as Schiff bases are synthesized by the condensation of primary amines with carbonyl groups (Shi *et al*., 2007). These organic compounds find great utility in medicinal, agricultural, and cosmetic areas (Badawi *et al*., 2007; Zhu *et al*., 2003). They are known to exhibit very good antibacterial (Sridhar *et al*., 2001; Mladenova *et al*., 2002; Panneerselvam *et al*., 2005; Walsh *et al*., 1996; Pandeya *et al*., 1999a, b) antifungal, and antitumor activities (Liu *et al*., 1992; Hodnett and Dunn, 1970). Ha and Win, 2011 synthesized Schiff base esters from 4-formyl-3-hydroxyphenyl octadecanoate and 3-substituted-anilines which exhibited good thermocrystalline behavior.

A series of novel Schiff base cationic surfactants were prepared by Negm *et al*., (2011a) by quaternization of Schiff base-alkyl ketoglutarates. The title molecules exhibited good anticorrosion and as antibacterial biocides. Schiff bases derived from 5-chloro-salicylaldehyde by reacting with primary amines (Shi *et al*., 2007) exhibited potential antibacterial and antifungal activities. Novel eco-friendly Schiff bases vanillin derived cationic surfactants were prepared by Negm *et al*., (2011b). These molecules exhibited good surface-active behavior. Schiff base esters containing pyridine moiety were prepared and these molecules were studied for their crystal structure behavior (Rauf and Parveen, 2005).

With increasing number of diseases and alarming problems of microbial resistance to antibiotics, the discovery of novel bioactive compounds against targeted diseases has been the need of the hour. In the recent years, plant-based renewable resources have gained increased attention. Fatty Acids and their derivatives were found to exhibit good antimicrobial and antifungal agents (Rauf and



Scheme 1 Synthesis of Schiff base



Scheme 2 Synthesis of Schiff base esters. **R** = C_6H_{13} (**2a**), C_7H_{15} (**2b**), C_9H_{19} (**2c**), $C_{11}H_{23}$ (**2d**), $C_{13}H_{27}$ (**2e**), $C_{15}H_{31}$ (**2f**), $C_{17}H_{35}$ (**2g**), *Sterculia foetida* (**2h**), coconut (**2i**), palm (**2j**), karanja (**2k**) fatty acids

Parveen, 2005; Sharma *et al*., 2005). Researchers have synthesized a wide variety of Schiff base esters (Ha *et al*., 2010) and most of them exhibited good antimicrobial properties. Long chain fatty acids containing Schiff bases with a polar head group exhibit good surface-active behavior. However, the fatty acid based alkanoyloxy Schiff base esters were not studied for antimicrobial and anticancer studies. Keeping this in view, we synthesized a series of different fatty acid Schiff base esters from pure and different fatty acid mixtures of vegetable oil origin. These esters were screened for antimicrobial and anticancer activities. The aim of the present study is to check the effect of alkanoyloxy chain length and nature of fatty acid mixtures prepared from different vegetable oils attached to Schiff base on the antimicrobial and anticancer activities.

Results and discussion

Chemistry

The Schiff base esters of different fatty acids (**2a–k**), eleven Schiff base esters were prepared from different fatty acids. The fatty acids with varied chain lengths were chosen for the study. The Schiff base esters were prepared in a two step procedure. 4-hydroxy benzaldehyde and 3-aminomethylpyridine in ethanol were reacted to obtain Schiff base shown in Scheme 1. The Schiff base (**1**) was reacted with fatty acids to obtain the Schiff base esters **2a–k** shown in Scheme 2. All the synthesized fatty Schiff base esters were characterized by GC–MS, ^{1}H NMR, ^{13}C NMR, and ESI–MS. The synthesized Schiff base esters were screened for antimicrobial and anticancer activities.

Different Schiff base compounds with different alkanoyloxy chain lengths have varied behavior on antibacterial, antifungal, and anticancer activities. In the present study, we focused on a series of synthesized Schiff base esters with different alkanoyloxy chain lengths. The fatty acids used were shorter chain pure fatty acids (**2a–c**), longer chain fatty acids (**2d–g**), and mixture of fatty acids (**2h–k**) prepared from different vegetable oils such as *Sterculia foetida* oil (containing cyclopropene-rich fatty acids), coconut oil (containing medium chain-rich fatty acids), palm oil (containing saturated-rich fatty acids), and karanja oil (containing unsaturated-rich fatty acids).

The Schiff base esters (**2**) studied exhibited moderate antibacterial activity which ranged from 75 to 300 μg/ml as compared to neomycin (18.75 μg/ml) as standard. However, the compounds with shorter chain, namely heptanoyloxy (**2a**, 37.5 μg/ml), octanoyloxy (**2b**, 37.5 μg/ml), decanoyloxy (**2c**, 37.5 μg/ml) chain lengths exhibited good activity as compared to the parent compound Schiff base (**1**, 75 μg/ml), other long chain (**2d–g**) and fatty acid mixture based Schiff base esters (**2h–k**) (Table 1). Similar trend was observed by Galbraith *et al*., (1971); Kabara *et al*., (1972); and Feldlaufer *et al*., (1993) in their studies on the effect of fatty acid chain length on antimicrobial activity. However, none of the compounds exhibited activity against *Micrococcus luteus* MTCC 2470, *Escherichia coli* MTCC 739 and *Klebsiella planticola* MTCC 530 strains.

All Schiff base esters (**2a–k**) exhibited moderate antifungal activity as compared to fluconazole as standard. However, the shorter chain compounds **2a** (75 μg/ml) and **2c** (75 μg/ml) exhibited good activity against *Candida aaseri* MTCC 1962 (75 μg/ml), *Candida albicans* MTCC 3018 (75 μg/ml), *Issatchenkia hanoiensis* MTCC 4755 (75 μg/ml) as compared to standard fluconazole (32–64 μg/ml). The heptanoyloxy Schiff base ester (**2a**) exhibited extraordinary activity (75 μg/ml) against *Issatchenkia orientalis* MTCC 3020 (128 μg/ml). Kabara *et al*., (1972) and Bergsson *et al*., (2001) also observed similar effect of short chain fatty acids on antifungal activity. While, other compounds (**2b**, **2e**, **2h**, and **2k**) showed fairly moderate activity (75–300 μg/ml) as compared to the standard (16–128 g/ml) (Table 2). However, the compounds (**1**, **2d**, **2f–g,** and **2i–j**) did not show any activity against the tested *Candida* strains. Overall, the shorter chain Schiff base esters

Table 1 Anti-bacterial activities of Schiff base (**1**) and synthesized compounds (**2a–k**)

Tested strains	Minimum inhibitory concentration (MIC, μg/ml)												
	1	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	Neomycin
Staphylococcus aureus MTCC 96	75	37.5	37.5	37.5	150	75	–	300	75	75	75	300	18.75
Staphylococcus MLS16 MTCC 2940	–[a]	150	300	300	150	75	–	300	300	150	300	300	18.75
Bacillus subtilis MTCC 121	–	300	300	–	–	–	–	–	–	–	–	–	18.75
Pseudomonas aeruginosa MTCC 2453	–	37.5	300	75	–	–	–	300	–	300	75	–	18.75

– [a] No activity

Table 2 Antifungal activities of synthesized compounds (**2a–c**, **2e**, **2h**, **2k**)

Test compounds	Minimum inhibitory concentration (μg/ml)						
	2a	**2b**	**2c**	**2e**	**2h**	**2k**	Fluconazole
Candida albicans MTCC 227	75	150	300	300	300	300	32
Candida albicans MTCC 854	300	–[a]	300	300	–	300	32
Candida albicans MTCC 7315	150	150	300	300	300	300	32
Candida albicans MTCC 183	300	150	300	300	300	300	32
Candida albicans MTCC 3958	150	150	300	300	300	300	64
Candida albicans MTCC 1637	150	–	300	300	300	300	64
Candida albicans MTCC 3018	75	300	75	300	300	300	32
Candida albicans MTCC 3017	–	–	–	300	–	–	64
Candida parapsilosis MTCC 1744	300	–	150	–	150	150	16
Candida glabrata MTCC 3019	–	150	150	–	150	–	64
Candida aaseri MTCC 1962	75	300	75	–	–	–	64
Issatchenkia orientalis MTCC 3020	75	150	150	–	300	–	128
Issatchenkia hanoiensis MTCC 4755	75	300	75	–	150	150	64

[a] No activity

Table 3 Anti-cancer activities of Schiff base (**1**) and synthesized compounds

Compounds	IC_{50} (μM)	
	MDA-MB-231	MCF7
1	16.9	12
2a	14.96	–[a]
2b	15.80	12
2d	18.9	17.6
2e	26.61	–
2g	11.5	–
2i	17.2	–
Doxorubicin	<1	1

[a] No activity

(**2ac**) can be well- exploited for antibacterial and antifungal formulations.

The Schiff base esters studied (**2a–k**) were also screened against different human tumor cell lines, namely, HeLa derived from human cervical cancer cells (ATCC No. CCL-2), HepG2 derived from human hepatocellular liver carcinoma cells (ATCC No. HB-8065), A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), MDA-MB-23 derived from human breast adenocarcinoma cells (ATCC No. HTB-26), MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) and Neuro2a derived from mouse neuroblastoma cells (ATCC No. CCL-131). Interestingly, the shorter and medium chain Schiff base esters **1**, **2a**, **2b**, **2d**, **2e**, **2g**, **2i** exhibited cytotoxicity selectively against MDA-MB-231, while the parent compound (**1**), octanoyloxy (**2b**), and dodecanoyloxy (**2d**) based Schiff base esters exhibited cytotoxicity against MCF7 (Table 3). However, the tested compounds did not exhibit any cytotoxicity against other tested cell lines.

Conclusions

In conclusion, a series of fatty acid-based Schiff base esters were prepared with varied chain lengths and the effect of

fatty acyl groups on antimicrobial and anticancer activities was studied. Schiff base esters with shorter chain heptanoic (**2a**) and decanoic acid (**2c**) showed good activity against the tested bacterial and fungal strains. Schiff base esters with shorter (**2a**, **2b**) and medium chain fatty acids (**2d**, **2i**) exhibited good anticancer activity, selectively toward human breast adenocarcinoma cell lines, MDA-MB-231 and MCF7 and long chain fatty acid (**2g**) exhibited good anticancer activity selectively toward MDA-MB-231 as compared to Schiff base (**1**).

Experimental section

Chemistry

Different chemicals such as 4-hydroxybenzaldehyde, 3-aminomethylpyridine, dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), acetonitrile, absolute ethanol (reagent grade) were procured from different commercial suppliers like Sigma-Aldrich, St. Louis, MO, USA, S.D. Fine Chemicals Limited, Mumbai, India and Avra Chemicals Pvt. Ltd, Hyderabad, India. Pure fatty acids namely heptanoic, octanoic, decanoic, dodecanoic, myristic, palmitic, and stearic acids were purchased commercially from S.D. Fine Chemicals Limited, Mumbai, India. Mixture of fatty acids were obtained from vegetable oils namely coconut, palm and karanja oils purchased locally. *S. foetida* fatty acids were obtained from *S. foetida* oil extracted from *S. foetida* seeds collected from IICT Campus. The fatty acid composition of the mixture of fatty acids was determined by GC chromatographic methods. Melting points were measured by open capillary tubes on Barnstead Electrothermal apparatus. The ^{1}H NMR spectra were recorded at Varian 300 MHz and Innova 500 and 75 MHz for ^{13}C NMR and TMS was used as an internal standard. All the compounds were dissolved in $CDCl_3$ while, the parent Schiff base (**1**) was dissolved in DMSO-d_6 and the chemical shifts were recorded at δ values in parts per million (ppm) and tetramethylsilane was used as internal standard. ESI–MS spectra were recorded on Waters e2695 Seperators module (Waters, Milford, MA, USA) mass spectrometer.

*General procedure for the synthesis of Schiff base {4-[(pyridine-3-methylimino) methyl phenol} (**1**)*

4-[(Pyridine-3-ylmethylimino)methyl]phenol was prepared by taking equimolar ratio of 4-hydroxybenzaldehyde (0.1 mol) and 3-aminomethyl pyridine (0.1 mol) dissolved in 100 ml absolute ethanol in a 250 ml round bottom flask and stirred at 80–85 °C for 6 h magnetically (Scheme 1). The progress of the reaction was monitored using TLC eluted with 5 % methanol-chloroform as solvent system. The reaction was cooled and the reaction mixture was poured into crushed ice with vigorous stirring. The product was filtered off and then washed with distilled water 2–3 times until it was free of the unreacted amine, followed by drying in a vacuum desiccator to get the Schiff base (80–85 % isolated yield).

Schiff base {4-[pyridine-3-ylmethylimino)-methyl]-phenol} (**1**) Melting point: 143.6 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm 4.72 (s, 2H, N–C$\underline{H}_2$–); 6.79 (d, 2H, $J = 8.49$ Hz, Ar–H); 7.26 (dd, 1H, $J = 7.9$ Hz, Ar–H); 7.57 (d, 2H, $J = 7.7$ Hz, Ar–H); 7.66 (d, 1H, $J = 3.58$ Hz, Ar–H); 8.30 (s, 1H, Ar–H); 8.42 (d, 1H, $J = 4.7$ Hz, Ar–H); 8.52 (s, 1H, C$\underline{H}$=N); 9.6 (s, 1H, O$\underline{H}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ/ppm 161.02, 159.15, 147.85, 146.76, 134.43, 130.9, 128.74, 126.13, 122.28, 114.51, 60.68; ESI–MS(*m*/*z*): 213.2 [M+H].

*General procedure for the synthesis of Schiff base ester {4-[(pyridine-3-ylmethylimino)methyl]phenyl alkanoate} (**2a–k**)*

4-[(Pyridine-3-ylmethylimino)methyl]phenyl alkanoate was prepared by taking Schiff base (4.7 mmol) dissolved in 2 ml DMF taken in a 100 ml round bottom flask. To this was added a solution of fatty acids (**2a–k**) (4.7 mmol) and 4-dimethylamino pyridine; DMAP (0.47 mmol) taken in dichloromethane; DCM (35 ml). The mixture was magnetically stirred in an ice bath at 0 °C. *N*,*N*′-dicyclohexylcarbodiimide; DCC (4.7 mmol) dissolved in 10 ml DCM was added drop wise while stirring in the ice bath for 30 min. The mixture was stirred at room temperature for 12–24 h (Scheme 2). The contents were filtered to remove the byproduct, dicyclohexylurea; DCU and the excess solvent were removed from the filtrate by evaporation. The crude product was dissolved in 50 ml of 50 % hexane–ethyl acetate and washed with saturated sodium bicarbonate solution and then concentrated. The crude product was taken in a separating funnel, dissolved in 50 % hexane–ethyl acetate and to this acetonitrile was added and shaken. The process was continued 2–3 times to get pure Schiff base ester with 60–75 % isolated yields.

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl heptanoate} (**2a**) ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.89 (t, 3H, $J = 6.98$ Hz, –C$\underline{H}_3$); 1.30 (m, 6H, CH_3–(C$\underline{H}_2$)$_3$–); 1.78 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.52 (t, 2H, $J = 7.0$ Hz, –C$\underline{H}_2$–COO); 4.78 (s, 2H, –C$\underline{H}_2$–N=CH–); 6.93 (d, 2H, $J = 8.8$ Hz, Ar–$\underline{H}$); 7.63 (dd, 1H, $J = 8.0$ Hz, Ar–H); 7.28 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.90 (d, 1H, $J = 8.5$ Hz, Ar–H); 8.35 (s, 1H, –C$\underline{H}$=N–); 8.51 (d, 1H, $J = 4.7$ Hz, Ar–H); 8.60 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 170.1, 162.03, 161.58, 149.29, 148.18, 135.42,

134.01, 129.78, 128.47, 123.32, 114.50, 62.18, 31.69, 28.99, 25.89, 22.53, 14.01; ESI–MS (*m/z*): 325 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl octanoate} ***(2b)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.90 (t, 3H, $J = 7.0$ Hz, –C$\underline{H}_3$); 1.31 (m, 8H, CH_3–(C$\underline{H}_2$)$_4$–); 1.42 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.56 (t, 2H, $J = 7.0$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}$2–N=CH–); 7.15 (d, 2H, $J = 9.0$ Hz, Ar–$\underline{H}$); 7.66 (dd, 1H, $J = 8.0$ Hz, Ar–H); 7.8 (d, 2H, $J = 8.0$ Hz, Ar–$\underline{H}$); 7.92 (d, 1H, $J = 8.0$ Hz, Ar–H); 8.41 (s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 5.0$ Hz, Ar–H); 8.56 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 169.0, 162.01, 161.42, 149.19, 148.14, 135.44, 135.04, 131.85, 129.74, 128.39, 123.28, 114.59, 114.41, 62.09, 31.69, 29.87, 29.08, 29.01, 28.88, 25.86, 13.97; ESI–MS (*m/z*): 338.8 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl decanoate} ***(2c)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.91 (t, 3H, $J = 7.0$ Hz, –C$\underline{H}_3$); 1.30 (m, 12H, CH_3–(C$\underline{H}_2$)$_6$–); 1.74 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.53 (t, 2H, $J = 7.0$ Hz, –C$\underline{H}_2$–COO); 4.79 (s, 2H, –C$\underline{H}$2–N=CH–); 7.11 (d, 2H, $J = 8.5$ Hz, Ar–$\underline{H}$); 7.63 (dd, 1H, $J = 8.0$ Hz, Ar–H); 7.76 (d, 2H, $J = 8.5$ Hz, Ar–$\underline{H}$); 7.8 (d, 1H, $J = 8.0$ Hz, Ar–H); 8.37(s, 1H, –C$\underline{H}$=N–); 8.48 (d, 1H, $J = 4.0$ Hz, Ar–H); 8.56 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm 169.1, 162.07, 149.19, 148.19, 135.44, 135.07, 131.88, 129.7, 123.31, 114.61, 114.44, 62.1, 31.37, 29.44, 29.16, 29.01, 28.98, 25.85, 22.54, 13.98; ESI–MS (*m/z*): 367 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl dodecanoate} ***(2d)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.7$ Hz, –C$\underline{H}_3$); 1.26 (m, 16H, CH_3–(C$\underline{H}_2$)$_8$–); 1.73 (quint, 2H, –C$\underline{H}$2–CH2–COO); 2.56 (t, 2H, $J = 7.3$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}$2–N=CH–); 7.16 (d, 2H, $J = 8.5$ Hz Ar–$\underline{H}$); 7.7 (dd, 1H, $J = 7.9$ Hz, Ar–H); 7.76 (d, 2H, $J = 8.5$ Hz, Ar–$\underline{H}$); 7.93 (d, 1H, $J = 8.3$ Hz, Ar–H); 8.40 (s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 4.1$ Hz, Ar–H); 8.60 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm 170.0, 162.01, 161.40, 149.18, 148.13, 135.42, 135.04, 131.81, 129.72, 123.26, 114.41; ESI–MS (*m/z*): 394.9 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl tetradecanoate} ***(2e)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.79$ Hz, –C$\underline{H}_3$);1.26 (m, 20H, CH_3–(C$\underline{H}_2$)$_{10}$); 1.76 (quint, 2H, –C$\underline{H}$2–CH2–COO); 2.56 (t, 2H, $J = 8.3$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}_2$–N=CH–); 7.15 (d, 2H, $J = 8.4$ Hz, Ar–$\underline{H}$); 7.68 (dd, 1H, $J = 7.7$ Hz, Ar–$\underline{H}$); 7.81 (d, 2H, $J = 8.4$ Hz, Ar–$\underline{H}$); 7.92 (d, 1H, $J = 8.3$ Hz, Ar–$\underline{H}$); 8.42 (s, 1H, –C$\underline{H}$=N–); 8.53 (d, 1H, $J = 4.1$ Hz, Ar–H); 8.60 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm 170.02, 162.07, 161.45, 149.24, 148.22, 135.50, 129.77, 123.24, 114.47, 62.16, 31.85, 29.52, 29.29, 29.09, 25.92, 22.60, 14.06; ESI–MS (*m/z*): 423 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl hexadecanoate} ***(2f)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.9$ Hz, –C$\underline{H}_3$); 1.26 (m, 24H, CH_3–(C$\underline{H}_2$)$_{12}$–); 1.61 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.56 (t, 2H, $J = 6.9$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}$2–N=CH–); 7.15 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.67 (dd, 1H, $J = 7.9$ Hz, Ar–$\underline{H}$); 7.92 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.92 (d, 1H, $J = 8.9$ Hz, Ar–$\underline{H}$); 8.41 (s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 4.9$ Hz, Ar–$\underline{H}$); 8.61 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 171.0, 162.13, 161.50, 149.23, 148.21, 135.53, 129.82, 123.36, 114.52, 62.17, 31.86, 29.64, 29.12, 25.95, 22.63, 18.37, 14.07; ESI–MS (*m/z*): 451 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl octadecanoate} ***(2g)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.9$ Hz, –C$\underline{H}_3$); 1.27 (m, 28H, CH_3–(C$\underline{H}_2$)$_{14}$–); 1.61 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.55 (t, 2H, $J = 6.9$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}_2$–N=CH–); 7.15 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.64 (dd, 1H, $J = 7.9$ Hz, Ar–$\underline{H}$); 7.80 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.91 (d, 1H, $J = 8.9$ Hz, Ar–$\underline{H}$); 8.41 (s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 4.9$ Hz, Ar–$\underline{H}$); 8.61 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 170.0, 162.07, 161.45, 149.24, 148.22, 135.51, 135.10, 131.91, 129.77, 128.44, 123.34, 114.47, 62.16, 31.85, 29.61, 29.29, 29.09, 25.92, 22.60, 14.05; ESI–MS (*m/z*): 479 [M+H].

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl ester of S. foetida fatty acids} ***(2h)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.5$ Hz, –C$\underline{H}_3$); 0.7 (s, 2H, cyclopropene ring); 1.26 (m, CH_3–(C$\underline{H}_2$)$_n$–); 1.62 (m, 2H, –C$\underline{H}_2$–CH_2–COO); 2.02 (t, 2H, methylene protan attached to cyclopropenoid ring); 2.35 (t, 2H, $J = 7.5$ Hz, –C$\underline{H}_2$–COO); 4.82 (s, 2H, –C$\underline{H}_2$–N=CH–); 7.11 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.67 (dd, 1H, $J = 7.9$ Hz, Ar–$\underline{H}$); 7.91 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.94 (d, 1H, $J = 8.9$ Hz, Ar–$\underline{H}$); 8.41 (s, 1H, –C$\underline{H}$=N–); 8.51 (d, 1H, $J = 4.9$ Hz, Ar–$\underline{H}$); 8.61 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 169, 162.11, 161.70, 149.21, 148.32, 135.62, 135.52, 134.5, 133.7, 131.02, 129.84, 123.38, 114.52, 59.32, 36.43, 34.23, 31.82, 29.63, 29.89, 29.12, 26.13, 23.60, 14.02.

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl ester of coconut fatty acids} ***(2i)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 6.9$ Hz,–C$\underline{H}_3$); 1.26 (m, CH_3–(C$\underline{H}_2$)$_n$–); 1.61 (quint, 2H, –C$\underline{H}_2$–CH_2–COO); 2.56 (t, 2H, $J = 6.9$ Hz, –C$\underline{H}_2$–COO); 4.80 (s, 2H, –C$\underline{H}$2–N=CH–); 5.36 (m, 2H, –C$\underline{H}$=CH–); 7.16 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.68 (dd, 1H, $J = 7.9$ Hz, Ar–$\underline{H}$); 7.92 (d, 2H, $J = 8.9$ Hz, Ar–$\underline{H}$); 7.94 (d, 1H, $J = 8.9$ Hz, Ar–$\underline{H}$); 8.41(s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 4.9$ Hz, Ar–$\underline{H}$); 8.60 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 169.1, 162.17, 161.89, 149.25, 148.32, 135.67, 134.52, 133.9, 131.02, 129.84, 123.48, 114.53, 63.32, 34.23, 31.92, 29.55, 29.63, 29.89, 28.12, 26.12, 23.60, 14.03.

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl ester of palm fatty acids} ***(2j)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.89 (t, 3H, $J = 7.0$ Hz, –C$\underline{H}_3$); 1.25 (m, CH_3–(C$\underline{H}_2$)$_n$–); 1.77 (quint, 2H, –C$\underline{H}_2$–C$\underline{H}_2$–COO); 2.56 (t, 2H, $J = 7.0$ Hz, –C$\underline{H}_2$–COO); 4.81 (s, 2H, –C$\underline{H}$2–N=CH–); 5.35 (m, 2H, –C$\underline{H}$=CH–); 7.15 (d, 2H, $J = 9.0$ Hz, Ar–$\underline{H}$); 7.67 (dd, 1H, $J = 8.0$ Hz, Ar–$\underline{H}$); 7.80 (d, 2H, $J = 9.0$ Hz, Ar–$\underline{H}$); 7.92 (d, 1H, $J = 8.9$ Hz, Ar–$\underline{H}$); 8.41(s, 1H, –C$\underline{H}$=N–); 8.52 (d, 1H, $J = 3.0$ Hz, Ar–$\underline{H}$); 8.61 (s, 1H, Ar–$\underline{H}$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 170.01, 162.11, 161.70, 149.21, 148.32, 135.62, 135.52, 133.9, 131.02, 129.84, 123.38, 114.52, 62.32, 34.23, 31.92, 29.63, 29.89, 29.12, 26.12, 23.60, 14.02.

{4-[(Pyridine-3-ylmethylimino)-methyl]-phenyl ester of karanja fatty acids} ***(2k)*** ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm 0.88 (t, 3H, $J = 7.0$ Hz, –C$\underline{H}_3$,); 1.28 (m, 6H, CH_3–(C$\underline{H}_2$)$_3$–); 1.74 (quint, 2H, –C$\underline{H}_2$–C$\underline{H}_2$–COO); 2.54 (t, 2H, $J = 7.0$ Hz, –C$\underline{H}_2$–COO); 4.80 (s, 2H, –C$\underline{H}$2–N=CH–); 5.34 (m, 2H, –C$\underline{H}$=CH–); 7.16 (d, 2H, $J = 9.0$ Hz, Ar–$\underline{H}$); 7.63 (dd, 1H, $J = 8.0$ Hz, Ar–H); 7.78 (d, 2H, $J = 8.5$ Hz, Ar–$\underline{H}$); 7.9 (d, 1H, $J = 8.9$ Hz, Ar–H); 8.40 (s, 1H, –C$\underline{H}$=N), 8.53 (d, 1H, $J = 4.0$ Hz, Ar–$\underline{H}$); 8.56 (s, 1H, Ar–$\underline{H}$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm 169.02, 162.11, 161.71, 149.25, 148.32, 135.67, 134.52, 133.9, 131.02, 129.84, 123.38, 114.55, 63.32, 34.23, 31.92, 29.55, 29.63, 29.89, 29.12, 26.12, 23.60, 14.1.

Biological evaluation

Assay for antibacterial and antifungal activities: The antibacterial and antifungal activities of the Schiff base (**1**) and synthesized esters (**2**) were determined using modified microtiter broth dilution method (Amsterdam, 1996) against different pathogenic bacterial reference strains like *Bacillus subtilis* MTCC 2940, *Staphylococcus aureus* MTCC 96, *S. aureus* MLS16 MTCC 2970, *M. luteus* MTCC 2470, *E. coli* MTCC 739, *K. planticola* MTCC 530, and *Pseudomonas aeruginosa* MTCC 2453 and pathogenic *Candida* reference strains such as *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 7315, *C. albicans* MTCC 183, *C. albicans* MTCC 3958, *C. albicans* MTCC 1637, *C. albicans* MTCC 3017, *C. albicans* MTCC 3018, *Candida parapsilosis* MTCC 1744, *Candida glabrata* MTCC 3019, *C. aaseri* MTCC 1962, *I. orientalis* MTCC 3020 and *I. hanoiensis* MTCC 4755 procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The bacterial and *Candida* reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the test compounds were loaded at a dose range of 300–1.4 μg well^{-1} in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of neomycin (antibacterial) and fluconazole (antifungal) at a dose range of 300–1.4 μg well^{-1} and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 30 °C and the well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

Cytotoxicity assay

Cytotoxicity of the Schiff base (**1**) and synthesized esters (**2**) was determined on the basis of measurement of in vitro growth inhibition of tumor cell lines in 96-well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using doxorubicin as a standard. The cytotoxicity as assessed against a panel of four different human tumor cell lines: A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No. HTB-26) and MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) using the MTT assay (Mosmann, 1983). The IC_{50} values (50 % inhibitory concentration) were calculated from the plotted absorbance data for the dose–response curves. IC_{50} values (in μM) are expressed as the average of two independent experiments.

Acknowledgments One of the author Y. M. thanks to the Director, Indian Institute of Chemical Technology, Hyderabad, India and thanks to the Head, Centre for Lipid Research, IICT for giving the financial support.

References

Amsterdam D (1996) Susceptibility testing of antimicrobials in liquid media. In: Lorian V (ed) Antibiotics in laboratory medicine, 4th edn. Williams and Wilkins, Baltimore

Badawi AH, Mohamed MAS, Mohamed MZ, Khowdairy MM (2007) Surface and anti tumor activity of some novel metal-based cationic surfactants. Assoc Radiat Oncol India 3:198–206

Bergsson G, Arnfinnsson J, Steingrimssomsson O, Thormar H (2001) Killing of Gram-positive cocci by fatty acids and monoglycerides. APMIS 109:670–678

Feldlaufer MF, Knox DA, Lusby WR, Shimanuki H (1993) Antibacterial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease. Apidologie 24:95–99

Galbraith H, Miller TB, Paton AM, Thampson JK (1971) Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. J Appl Bacteriol 34:803–813

Ha S-T, Win Y-F (2011) Synthesis, spectral studies of 4-{[(3-substitutedphenyl)imino]methyl}-3-hydroxyphenyl octadecanoate and effect of meta substituents on mesomorphic properties. Int J Phy Sci 6:2507–2517

Ha S-T, Ong L-K, Win Y-F, Yeap G-Y, Bonde NL, Boey P-L (2010) New Schiff bases with pyridine core: spectra, thermal and optical characterizations. J Am Appl Sci 7:656–660

Hodnett EM, Dunn JW (1970) Structure-anti-tumor activity correlation of some Schiff bases. J Med Chem 13:768–770

Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP (1972) Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother 2:23–28

Liu MC, Lin TS, Sartorelli AC (1992) Synthesis and antitumor activity of amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone. J Med Chem 35:3672–3677

Mladenova R, Ignatova M, Manolova N, Petrova T, Rashkov I (2002) Preparation, characterization and biological activity of Schiff base compounds derived from 8-hydroxyquinoline-2-carboxaldehyde and Jeffamines. Eur J Polym 38:989–999

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Negm NA, El Farargy AF, Al Sabagh AM, Abdelrahman NR (2011a) New Schiff base cationic surfactants: surface and thermodynamic properties and applicability in bacterial growth and metal corrosion prevention. J Surfactants Deterg 14:505–514

Negm NA, Kandile NG, Mohamad MA (2011b) Synthesis, characterization and surface activity of new eco-friendly Schiff bases vanillin derived cationic surfactants. J Surfactant Deterg 14: 325–331

Pandeya SN, Sriram D, Nath G, DeClercq E (1999a) Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and *N*-[4-(49-chlorophenyl)thiazol-2-yl] thiosemicarbazide. Eur J Phramacol 9:25–31

Pandeya SN, Sriram D, Nath E, DeClercq E (1999b) Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino-2-methylmercapto quinazolin-4(3H)-one. Pharm Acta Helv 74:11–17

Panneerselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK (2005) Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. Eur J Med Chem 40:225–229

Rauf A, Parveen H (2005) Synthesis and characterization of a new Schiff base and its metal complexes. Indian J Chem B 44: 1273–1276

Sharma S, Gangal S, Rauf A (2005) Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidin-4-ones and thiazan-4-one from 10-undecenoic acid hydrazide. Eur J Med Chem 40:173–184

Shi L, Ge H-M, Tan S-H, Li H-Q, Song Y-C, Zhu H-L, Tan R-X (2007) Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro salicylaldehyde. Eur J Med Chem 42:558–564

Sridhar SK, Saravanan M, Ramesh A (2001) Synthesis and antibacterial screening of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur J Med Chem 36:615–625

Walsh OM, Meegan MJ, Prendergast RM, Nakib TA (1996) Synthesis of 3-acetoxyazetidin-2-ones and 3-hydroxyazetidin-2-ones with antifungal and antibacterial activity. Eur J Med Chem 31: 989–1000

Zhu WR, Hu PZ, Li MY, Huang XL, Wu CT (2003) Synthesis of new Schiff bases containing thiophene moiety. J Nat Sci 8:433–436