Journal of Organometallic Chemistry 694 (2009) 2198–2203

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Synthesis and biological evaluations of some Schiff-base esters of ferrocenyl aniline and simple aniline

Haq Nawaz [a], Zareen Akhter [a,*], Sumaira Yameen [a], Huamaira M. Siddiqi [a], Bushra Mirza [b], Arshia Rifat [b]

[a] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[b] Department of Biochemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

ARTICLE INFO

Article history:
Received 24 November 2008
Received in revised form 18 February 2009
Accepted 24 February 2009
Available online 9 March 2009

Keywords:
Schiff base
Schiff-base esters
Ferrocene
Ferrocenyl aniline
Biological activities

ABSTRACT

The synthesis and biological studies of some long chain esters containing Schiff bases and their ferrocenyl analogues were carried out. The 4-amino ferrocene was prepared by the reported method. Long chain esters were synthesized by the condensation of different aliphatic acids with the corresponding aldehyde. The esters were then reacted with aniline as well as with 4-aminophenyl ferrocene to give corresponding Schiff bases. All the synthesized compounds were analyzed by elemental, FTIR and proton NMR studies, were also investigated for a range of biological activities. Determined by crown gall tumor inhibition assay. Antioxidant and DNA protective activities were determined by DPPH free radical scavenging assay and OH radical induced oxidative DNA damage assay, respectively. Among all test compounds, *o*-hydroxy-*p*-*n*-octadecanoyloxy-benzylidine-*p*-ferrocenyl aniline (FA2.1: a ferrocene containing Schiff base) showed highest antitumor, DPPH free radical scavenging and DNA protective activities.

1. Introduction

Drug discovery process involves synthesis of novel bioactive compounds which undergo different in vitro and in vivo testing before their clinical administration [1,2]. In last few years organometallics have got great importance owing to their potential biological activities [3,4]. Among organometallics, ferrocene is known as chemically and thermally stable specie. The greater stability of ferrocene system led to its incorporation in many research activities. Nowadays, ferrocene is no longer a chemical curiosity, rather it provides a widely applicable platform for the preparation of functional derivatives used in many areas like catalysis, material science, crystal engineering and bio-organometallic chemistry [5–8]. The activities of certain drugs are reported to be enhanced by the addition of ferrocene moiety to their structures; e.g., ferrocene aspirin [4], antimalarial drug ferrocene quine [9], an anticancer drug ferrocifen [10], ferrocene–hydroxytamoxifen [11] and ferrchloroquine [12]. The anticancer potential of ferrocene containing compounds is also well reported [13–16].

Keeping in view the reported biological studies, simple Schiff bases (AN1, AN2, AN1.1 and AN2.1), Schiff bases with ferrocene addition (FA1, FA2, FA1.1 and FA2.1) and their reactants (Z1, Z1.1, A1, A2, A1.1 and A2.1) were investigated for their differential biological activities towards antitumor, antioxidant and DNA protecting effects. In the present study crown gall tumor inhibition assay is used for verification of anticancerous activity of these Schiff bases. The purpose of DPPH scavenging assay was to evaluate the antioxidative potential of test samples. While in vitro OH radical induced DNA damage system served to evaluate DNA protection in oxidative stress.

2. Experimental

2.1. Materials

Ferrocene, 4-nitroaniline, sodium nitrate, hexadecyltrimethylammonium bromide, hydrochloric acid, aniline, hydrazine, dimethylaminopyridene, hexadecyltrimethyl ammonium bromide (PTC) *N*,*N*′-dicyclohexylcarbodiimide and 2,4-dihydroxybenzaldehyde were obtained from Fluka, Switzerland. Solvents such as dichloromethane, absolute ethanol, diethyl ether, hexane, ethyl acetate and petroleum ether were obtained from Merck Germany and freshly dried as per requirement using the standard methods.

2.2. Synthesis of 4-aminophenyl ferrocene

4-Ferrocenyl aniline was prepared in two steps by the reported method [5].

2.3. General procedure for the synthesis of long chain esters (A1, A1.1, A2 and A2.1)

In a two neck round bottom flak equipped with magnetic stirrer and reflux condenser, the corresponding aldehyde (10 mmol), long chain aliphatic acid (10 mmol) and dimethylaminopyridene

* Corresponding author. Tel.: +92 51 90642111; fax: +92 51 2873869.
E-mail address: zareenakhter@yahoo.com (Z. Akhter).

0022-328X/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2009.02.032

(1 mmol) were dissolved in 1:1 mixture of dimethylformamide and dichloromethane and stirred at 0 °C. To this stirred solution, a solution of *N*,*N*′-dicyclohexycarbodiimide (10 mmol) was added drop wise and stirred at 0 °C for 1 h and then stirred at room temperature for 3 h. The progress of the reaction was monitored by thin layer chromatography. After the completion of the reaction, the reaction mixture was filtered and DCM was removed from filtrate by rotary evaporator. The remaining mixture was poured in water and precipitated ester was filtered and recrystallized with *n*-hexane to get pure product [17–19].

2.4. General procedure for the synthesis of Schiff-bases esters (AN1, AN1.1, AN2, AN2.1, FA1, FA1.1, FA2 and FA2.1 [20]

In a pre-backed round flask (100 ml) equipped with stirrer and reflux condenser, the solution of corresponding long chain aromatic ester (2.5 mmol) in absolute ethanol was added. To this, the solution of corresponding aniline (2.5 mmol) in absolute ethanol was added drop wise with constant stirring. The reaction mixture was refluxed for 4–5 h. After the completion of the reaction, the ethanol was removed by rotary evaporator and the product was recrystallized from absolute ethanol.

2.4.1. P-n-hexadecananoyloxy-benzaldehyde-p-aniline (AN1)

Off white solid, yield (74%), m.p. 57–76 °C. Anal. Calc. for $C_{29}H_{41}O_2N$: C, 80.00; H, 9.42; N, 3.21. Found: C, 79.69; H, 9.58; N, 3.69%. IR (cm^{-1}) 1625, 1656, 1746, 2920, 2850. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.16^s; δ (–CH_3) = 1.03^t; δ (–CH_2COO–) = 2.37^t: δ (–CH_2–) = 1.75^m, 1.35^m; δ (Ar–H) = 7.80^m, 7.10^m.

2.4.2. O-hydroxy-p-n-hexadecananoyloxy-benzaldehyde-p-aniline (AN1.1)

Yellow solid, yield (75%), m.p. 78–94 °C. Anal. Calc. for $C_{29}H_{41}O_3N$: C, 77.16; H, 9.09; N, 3.10. Found: C, 77.40; H, 9.83; N, 3.38%. IR (cm^{-1}) 1629, 1655, 1756, 2917, 2849, 3311. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.63^s; δ (Ar–OH) = 13.60^s; δ (–CH_3) = 0.92^t; δ (–CH_2COO–) = 2.58^t: δ (–CH_2–) = 2.30^m, 1.27^m; δ (Ar–H) = 7.47^m, 7.28^m.

2.4.3. P-n-octadecananoyloxy-benzaldehyde-p-aniline (AN2)

Light yellow solid, yield (73%), m.p. 62–78 °C. Anal. Calc. for $C_{31}H_{45}O_2N$: C, 80.34; H, 9.71; N, 3.02. Found: C, 79.02; H, 10.00; N, 3.16%. IR (cm^{-1}) 1560, 1657, 1749, 2918, 2850. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.17^s; δ (–CH_3) = 1.02^t; δ (–CH_2COO–) = 2.37^t: δ (–CH_2–) = 1.75^m, 1.20^m; δ (Ar–H) = 7.86^m, 7.14^m.

2.4.4. O-hydroxy-p-n-octadecananoyloxy-benzaldehyde-p-aniline (AN2.1)

Light yellow solid, yield (75%), m.p. 62–80. Anal. Calc. for $C_{31}H_{45}O_3N$: C, 77.66; H, 9.39; N, 2.92. Found: C, 77.02; H, 9.89; N, 3.04%. IR (cm^{-1}) 1576, 1691, 1759,2917, 2850, 3400. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.67^s; δ (Ar–OH) = 13.62^s δ (–CH_3) = 0.91^t; δ (–CH_2COO–) = 2.37^t: δ (–CH_2–) = 2.60^m, 1.14^m; δ (Ar–H) = 7.28^m, 6.72^m.

2.4.5. P-n-hexadecanoyloxy-benzylidine-p-ferrocenyl aniline (FA1)

Orange solid, yield (70%), m.p. 97–110 °C. Anal. Calc. for $C_{39}H_{49}O_2NFe$: C, 74.60; H, 7.91; N, 2.26. Found: C, 74.52; H, 8.44; N, 2.62%. IR (cm^{-1}) 499, 1605, 1622, 1755, 2850, 2917. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.32^s; δ (–CH_3) = 1.03^t; δ (–CH_2COO–) = 2.38^t: δ (–CH_2–) = 1.45^m, 1.36^m; δ (Ar–H) = 7.95^m, 7.55^m, δ (–C_5H_5) = 4.02^s: δ (–C_5H_4–) = 4.27^t; δ (–C_5H_4–) = 4.65^t.

2.4.6. P-n-octadecanoyloxy-benzylidine-p-ferrocenyl aniline (FA2)

Orange solid, yield (72%), m.p. 114–125 °C. Anal. Calc. for $C_{41}H_{53}O_2NFe$: C, 76.04; H, 8.19; N, 2.16. Found: C, 75.48; H, 8.23; N, 2.28%. IR (cm^{-1}) 507, 1605, 1631, 1754, 2850, 2818. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.32^s; δ (–CH_3) = 1.03^t; δ (–CH_2COO–) = 2.38^t: δ (–CH_2–) = 1.76^m, 1.36^m; δ (Ar–H) = 7.33^m, 7.96^m, δ (–C_5H_5) = 4.04^s: δ (–C_5H_4–) = 4.27^t; δ (–C_5H_4–) = 4.65^t.

2.4.7. O-hydroxy-p-n-hexadecanoyloxy-benzylidine-p-ferrocenyl aniline (FA1.1)

Orange solid, yield (74%), m.p. 123–138 °C. Anal. Calc. for $C_{39}H_{49}O_3NFe$: C, 73.70; H, 7.72; N, 2.20. Found; C, 72.49; H, 7.49; N, 2.45%. IR (cm^{-1}) 493, 1601, 1621, 1754, 2849, 2918. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.16^s; δ (–CH_3) = 1.01^t; δ (–CH_2COO–) = 2.34^t: δ (–CH_2–) = 1.5^m, 1.03^m; δ (Ar–H) = 7.48^m, δ (Ar–OH) = 14.09^s; δ (–C_5H_5)^2 = 3.98^s: δ (–C_5H_4–) = 4.27^t; δ (–C_5H_4–) = 4.62^t.

2.4.8. O-hydroxy-p-n-octadecanoyloxy-benzylidine-p-ferrocenyl aniline (FA2.1)

Orange solid, yield (79%), m.p. 100–120 °C. Anal. Calc. for $C_{39}H_{49}O_3NFe$: C, 80.31; H, 7.84; N, 2.68. Found: C, 79.39; H, 7.60; N, 2.34%. IR (cm^{-1}) 503, 1601, 1622, 1756, 2850, 2922. ^1H NMR (DMSO-d_6, δ ppm): δ (–CH═N–) = 8.70^s; δ (–CH_3) = 0.91^t; δ (–CH_2COO–) = 2.35^t: δ (–CH_2–) = 2.58^m, 1.14^m; δ (Ar–H) = 7.48^m, δ (Ar–OH) = 13.80^s; δ (–C_5H_5) = 4.08^s: δ (–C_5H_4–) = 4.18^t; δ (–C_5H_4–) = 4.25^t.

2.5. Antitumor potato disc assay

Antitumor potato disc method was used for the determination of antitumor activities [21]. Briefly, 48-h-old single colony culture of Agrobactreium tumefaciens (At-10) was used as an agent for tumor induction on potato discs. Rifampicine (10 mg/ml) was used as a specificity drug for At-10. Each test sample was evaluated for antitumor activity at three concentrations i.e., 1000, 100 and 10 μg/ml with DMF as negative control and vincristine as standard drug. Under complete aseptic conditions sterilized instruments were used to make 8 × 5 mm potato discs from surface sterilized ($HgCl_2$ 0.1%) healthy potatoes tubers. Ten potato discs were placed on each plate containing 1.5% agar–agar in distilled water. After treatment with test agents and At-10 strain on each disc, plates were incubated (28 °C) for 21 days than stained with Lugol's solution (10% KI and 5% I2) and number of tumors was counted with the help of dissecting microscope. Each experiment was carried out in triplicate and data was statistically analyzed using ANOVA and Duncan's Multiple Range Test. Percentage tumor inhibition was calculated by using formula given in Table 1.

2.6. DPPH free radical scavenging assay

DPPH free radical scavenging assay was used to determine the free radical scavenging efficiency of test samples, as reported earlier [22]. DPPH is a stable free radical present in crystallized form. Reagents used for this assay were 0.1 mM DPPH in ethanol and 50 mM Tris–HCl in distilled water. Reaction mixture contained DPPH, Tris–HCl and test sample in fixed volume ratio of 2 ml, 900 μl and 100 μl, respectively. Final volume was kept in caped glass vials and incubated at room temperature for half an hour. Test samples were examined at 100, 50 and 25 μg/ml as final concentration from stock. Ascorbic acid was used as a positive control and DMF as negative control. Change in DPPH purple color was observed by spectrophotometric absorbance at 517 nm. Each experiment was performed in triplicate and data was analyzed using ANOVA and Duncan's Multiple Range test. Percentage free radical scavenging was calculated by using formula given in Table 2.

Table 1.
Antitumor activity of tested compounds.

Organic compounds	Percentage tumor inhibition ± S.E.			IC_{50} (μg/ml)	Organic compounds	Percentage tumor inhibition ± S.E.			IC_{50} (μg/ml)
	1000 (μg/ml)	100 (μg/ml)	10 (μg/ml)			1000 (μg/ml)	100 (μg/ml)	10 (μg/ml)	
$Z1^{CDE}$	29 ± 3.9	25 ± 1.2	9 ± 1.2	>1000	$AN2^{ABC}$	52 ± 7.7	34 ± 5.4	28 ± 5.6	900
$Z1.1^{CDE}$	27 ± 3.5	22 ± 3.2	13 ± 1.9	>1000	$AN1.1^{CDE}$	24 ± 11.1	18 ± 4.6	11 ± 2.3	>1000
$A1^{DE}$	19 ± 7.8	12 ± 2.6	3 ± 0.2	>1000	$AN2.1^{DE}$	29 ± 10	7 ± 2.7	6 ± 2	>1000
$A2^{ABCDE}$	55 ± 12	27 ± 11.4	14 ± 4	868	$FA1^{CDE}$	20 ± 15.5	15 ± 6.9	15 ± 1.2	>1000
$A1.1^{CDE}$	38 ± 8.6	3 ± 2.8	3 ± 0.3	>1000	$FA2^{CDE}$	30 ± 8.1	20 ± 2.1	18 ± 7.0	>1000
$A2.1^{E}$	19 ± 5.3	4 ± 1.2	1 ± 2.7	>1000	$FA1.1^{AB}$	58 ± 4.4	41 ± 6.5	40 ± 4.2	563
$AN1^{BCDE}$	38 ± 5.1	23 ± 7.7	17 ± 8.6	>1000	$FA2.1^{A}$	71 ± 3.5	40 ± 0.2	35 ± 4.8	20
−ve Control	–	–	–	–	Vincristine	100 ± 0	100 ± 0	100 ± 0	0.003

Percentage tumor inhibition $= \left(\frac{100 - \text{Average no of tumors in sample}}{\text{Average no of tumors in negative control}}\right) \times 100$.
- Standard drug vincristine was used as a positive control.
- More than 20% inhibition is significant.
- Data represents average percentage tumor inhibition of three replicates ± S.E.
- Letters ranging from A to E indicate least significant difference rank orders of percentage tumor inhibition. Values with similar letters are not significantly different from each other at $p < 0.05$.

2.7. OH radical induced oxidative DNA damage analysis

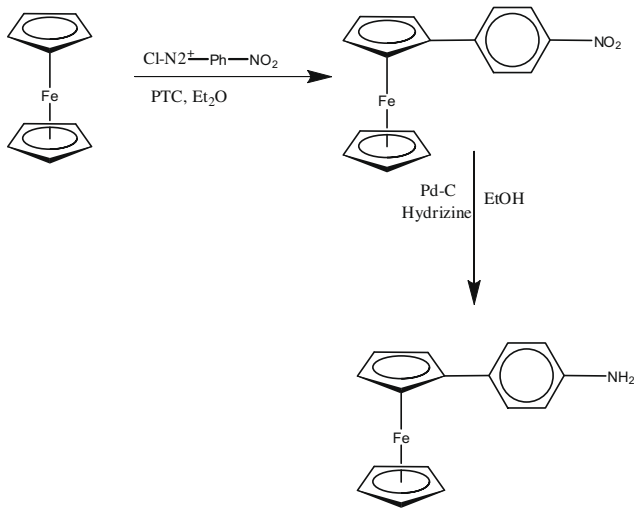
Study of protection of DNA by test samples was carried out as reported in vitro OH radical induced oxidative DNA damage assay [23]. Plasmid pBR322 DNA (Fermentas, Germany) was diluted by using 50 mM phosphate buffer pH 7.4 to get a concentration of 0.5 μg/3 μl. Reaction mixture (kept in eppendorf tube at a total volume of 15 μl) contained diluted pBR322 (0.5 μg/ml) test sample (10 μg/ml), 2 mM $FeSO_4$ and 30% H_2O_2. For each experiment a 1 Kb DNA ladder (L), a negative control (only plasmid DNA, P) and plasmid DNA treated with H_2O_2 and $FeSO_4$ as positive control (Fenton reaction mixture, X) were also used. Reaction mixture was incubated at 37 °C for 1 h in dark. Each test was subjected to 0.9% agarose gel electrophoresis in 1X TBE gel running buffer. Ethedium bromide stained gel was photographed under the UV light and analyzed by Lab Image 1D gel analysis software (2006).

3. Results and discussion

3.1. Synthesis

To prepare 4-aminophenyl ferrocene, 4-nitrophenyl ferrocene was first prepared through the arylation of ferrocene by the diazonium salt of 4-nitroaniline in the presence of phase transfer catalyst using ethanol as solvent. The 4-nitrophenyl ferrocene was then reduced to 4-aminophenyl ferrocene using 5% Pd/C and hydrazine as reducing agents and reaction mixture was refluxed for about 10–12 h (scheme 1) [5].

Long chain esters were prepared by reacting different long chain alkyl aliphatic acids with the corresponding aldehydes



Scheme 1. Synthesis of ferrocenyl aniline.

Table 2.
DPPH free radical scavenging activity of tested compounds.

Organic compounds	Percentage scavenging ± S.E.			IC_{50} (μg/ml)	Organic compounds	Percentage scavenging ± S.E.			IC_{50} (μg/ml)
	100 (μg/ml)	50 (μg/ml)	25 (μg/ml)			100 (μg/ml)	50 (μg/ml)	25 (μg/ml)	
$Z1^{EF}$	−6 ± 1.2	−5 ± 2.7	8 ± 0.6	–	$AN2^{D}$	16 ± 0.1	12 ± 0.3	8 ± 0.2	>100
$Z1.1^{E}$	−2 ± 2.2	1 ± 0.7	2 ± 0.7	–	$AN1.1^{A}$	77 ± 2.2	52 ± 1.4	36 ± 0.3	49.3
$A1^{E}$	−1 ± 0.7	−1 ± 1.4	2 ± 0.5	–	$AN2.1^{D}$	13 ± 1.6	11 ± 0.5	11 ± 0.5	>100
$A2^{FG}$	−3 ± 0.9	−5 ± 0.3	−1 ± 5.8	–	$FA1^{C}$	42 ± 0	36 ± 2.6	11 ± 2.6	>100
$A1.1^{H}$	−6 ± 0.7	−8 ± 2.6	−7 ± 0.5	–	$FA2^{B}$	44 ± 0	44 ± 0.6	30 ± 1.2	>100
$A2.1^{G}$	−11 ± 0.3	−4 ± 0.6	−5 ± 0.4	–	$FA1.1^{C}$	38 ± 1.8	30 ± 6.2	26 ± 1.2	174.1
$AN1^{E}$	1 ± 0.1	5 ± 0.4	4 ± 0.2	>100	$FA2.1^{A}$	65 ± 2.8	56 ± 1.3	41 ± 0.2	45.7
−ve Control	–	–	–	–	Ascorbic acid	70.3 ± 0	74 ± 0	81 ± 0	8.2

- Absorbance was taken at 517 nm spectrophotometrically.
- Tested samples were tested at final dilution of 100, 50 and 25 μg/ml.
- Commercial antioxidant ascorbic acid was used as a positive control.
- "–" represents pro-oxidant activities.
- Percentage tumor inhibition $= \left(\frac{\text{absorbance of negative controll-absorbance of sample}}{\text{Absorbance of negative control}}\right) \times 100$.
- Data represents average percentage scavenging of three replicates ± S.E.
- Least significant difference rank order on the basis of DPPH percentage scavenging is represented by letters ranging from A to H at $p < 0.05$.

(4-hydroxy benzaldehyde, 2,4-dihydroxybenzaldehyde) in the presence of DCC and DMAP (Scheme 2) using DCM and DMF as solvents. The products obtained on evaporating the solvent were recrystallized with *n*-hexane to get pure product.

Schiff-bases esters were synthesized according to the Scheme 2 given below in which corresponding aniline was refluxed with long chain esters in inert atmosphere using dry nitrogen and absolute ethanol as solvent.

4-Nitrophenyl ferrrocene and 4-aminophenyl ferrocene as well as all esters and ester containing Schiff bases were characterized by their characteristic color, melting points, CHNS, FTIR and proton NMR spectral data. The elemental analyses of all the products are in agreement with the calculated values. The I.R spectra of these products show all the characteristics peaks. Asymmetric and symmetric stretches of NO_2 in case of 4-aminophenyl ferrocene were observed at 1508 and 1339 cm^{-1}, respectively, which were absent in case of ferrocenyl aniline. The aromatic υ_{C-H} stretch in the ferrocene derivatives appeared at 2924 and 3092 cm^{-1}. Esters gave a strong band around 1700 cm^{-1} representing the stretching vibrations of the carbonyl group where as the esters containing hydroxyl group gave a broad band at 3327–3450 cm^{-1} due to υ_{O-H} stretch.

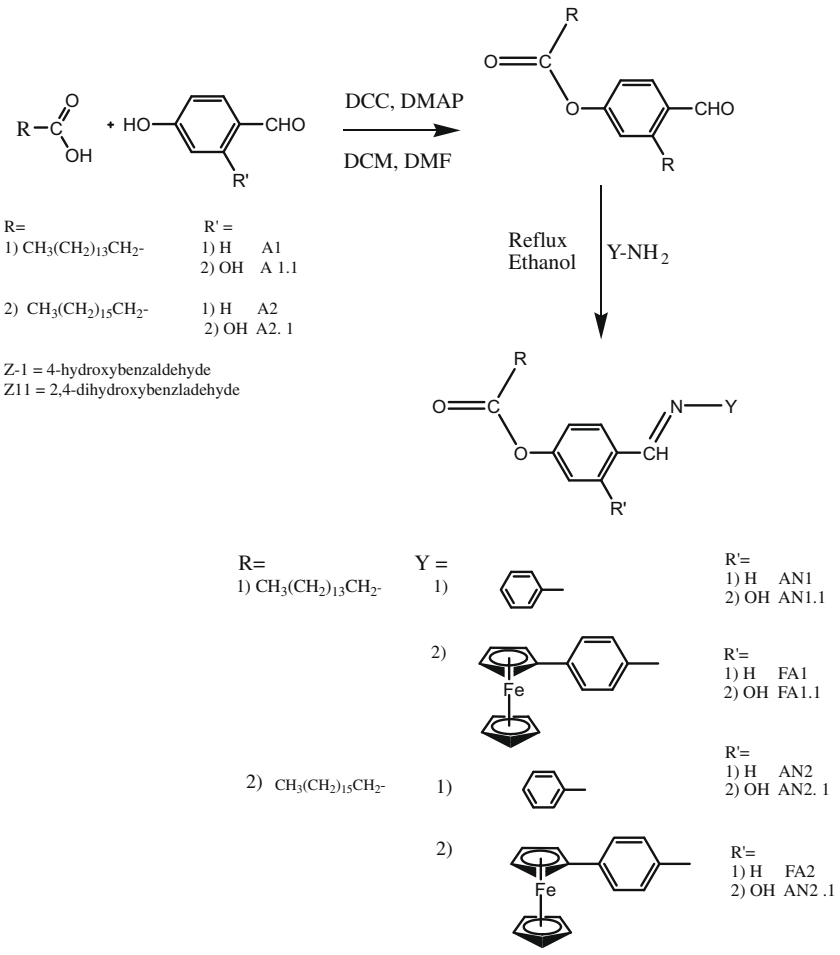
In case of schiff bases, the C=N stretch of azomethine group was observed at 1560–1627 cm^{-1} and in ferrocene based Schiff bases the Fe–Cp vibrations were observed at 493–507 and 924–1148 cm^{-1}. All the characteristics signals were observed in the ^1H NMR spectra of the synthesized Schiff bases. The incorporation of the ferrocene is indicated by the signals observed due to ferrocene. The unsubstituted cyclopentadienyl ring of ferrocene gave a singlet in the range 3.98–4.04 ppm for the five hydrogens and the substituted one gave the triplets at 4.27 ppm for the two hydrogens and at 4.62–4.65 ppm for the other two hydrogens in agreement with the literature values [24]. All the Schiff bases showed a sharp singlet around 8.16–8.63 ppm for azomethine proton in accordance with literature [24].

3.2. Biological activities

The biological study reported herein is accessed by using selected biological assays. This is an attempt to investigate possible biological activities of synthesized compounds and any possible change in activities followed by ferrocene addition.

3.2.1. Antitumor activity

Potato disc tumor induction assay is a general reliable screen for detecting antitumor agents [25–29]. Mechanisms for tumor induction are reported to be similar in plants and animals and potato disc assay had shown a good correlation in results to other most commonly used antitumor screening assays [30]. In present study Agrobacterium tumefaciens (At-10) was used to induce tumors on potato discs [31]. In each experiment vincristine as positive control and DMF as negative control were used for reference. As depicted by results in Table 1 DMF (negative control) have no interference with the activity of bacterium to induce tumors whereas Vincristine (positive control) showed 100% tumor inhibition at all concentrations tested. Potato disc tumor inhibition analysis of



Scheme 2. Synthesis of ferrocene containing Schiff bases.

synthesized organic compounds showed that all the test compounds inhibit tumor production for treatment of 1000, 100 and 10 μg/ml concentration at $p < 0.05$ (Table 1). Furthermore, the inhibition was observed in dose dependant manner with highest inhibition at 1000 μg/ml concentration. Ferrocene Schiff base (ferrocene being reported as anticrcinogen) FA2.1 exhibited the best activity with 71% followed by FA1.1 with 58% inhibition at 1000 μg/ml. Antitumor activity can also be judged by 50% inhibitory concentration (calculated by inhibition curves of three concentrations tested for each compound). Lower the IC 50 value more effective is the tested compound. An IC 50 calculation also favours FA2.1 and FA 1.1 (ferrocene based Schiff bases) to be more active as compared to simple Schiff bases and reactive compounds. By analyzing structure effectiveness on activities it could be inferred from these results that the ferrocene presence as well as OH group substitution to benzene ring might had resulted in enhancing antitumor activity of FA2.1 and FA1.1. This observed result was consistent with a previously reported study [32].

3.2.2. DPPH free radical scavenging (Antioxidant activity)[5]

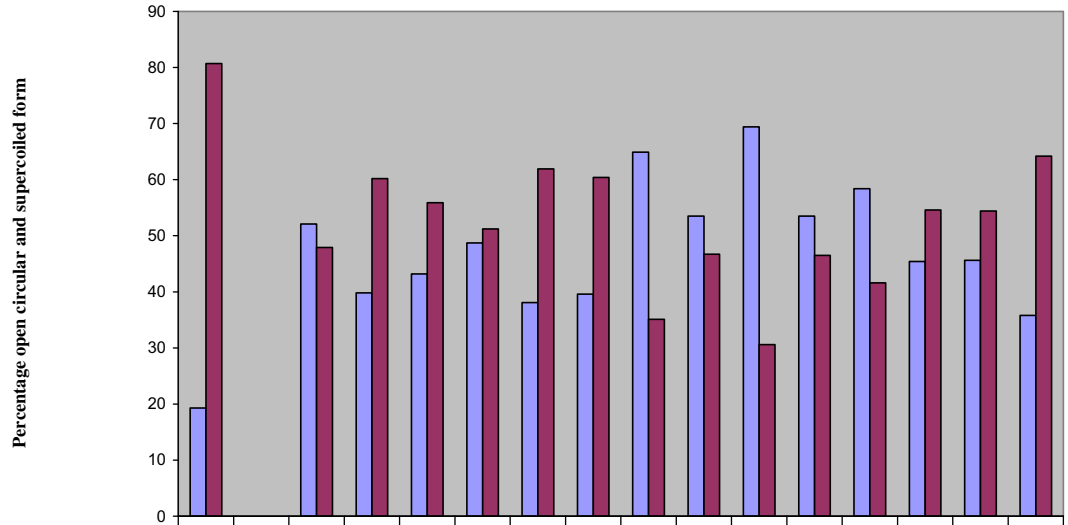
DPPH is a stable free radical. Antioxidants react with DPPH by donating electron or hydrogen thus neutralizing it to yellow color diphenyl picrylhydrazine [33]. This reduction of DPPH radical by antioxidants can be determined by the decrease in absorbance at 517 nm spectrophotometrically, however, an increase in absorbance will show pro-oxidant activities. DPPH free radical scavenging activities of Schiff bases were assayed at three concentrations 100, 50 and 25 μg/ml as shown in Table 2. Ascorbic acid was used as a positive control that showed DPPH discoloration. DPPH free radical scavenging activity of tested compound was concentration dependant. Higher radical scavenging was observed at higher concentration i.e. 100 μg/ml. DPPH scavenging analysis of compounds showed AN1.1 and FA2.1 are the best scavengers of DPPH among test agents by 77% and 65% scavenging activity respectively at 100 μg/ml final concentration. IC 50 (50% inhibitory concentration) calculations support FA2.1 to be best DPPH radical quencher with 45.7 μg/ml IC 50 value DPPH scavenging was observed in an order of ferrocene Schiff bases > Schiff bases, while no scavenging or pro-oxidant activities were shown by their reacting compounds (Z1, Z1.1, A1, A2, A1.1 and A2.1). Thus it could be assumed that turning of reacting compounds into Schiff bases and ferrocene addition to Schiff bases had resulted in increase in scavenging of free radicals. Similar enhancing free radical quenching activity by ferrocene addition was earlier reported [34].

3.2.3. OH radical induced oxidative DNA damage analysis

In a Fenton reaction, Fe^{2+} reacts with H_2O_2 and form OH radicals. OH radical is considered to be the most damaging to biomolecules [14,15]). DNA protecting activity of ferrocene sciff bases and other synthesized compounds were investigated by in vitro OH radical induced DNA damage system at 10 μg/ml final concentration. Normally pBR322 DNA exists in super-coiled form (SC). With the attack of OH radical generated from Fenton reaction SC was broken into open circular (OC). Data recorded in Fig. 1 depicts % SC form as DNA protecting activity and % OC form as DNA damaging activity of test agents.% SC form and % OC form were calculated by Lab Image ID gel analysis software (2006) for each compound. Negative control i.e., only pBR322 DNA showed given % SC form used in the test, while there is total DNA damage in positive control (X i.e., DNA + $FeSO_4$ + H_2O_2). Some reacting compounds like Z1.1, A1.1 and A2.1 showed greater than 60% DNA protecting activity. Among all the test compounds, FA2.1 had shown maximum DNA protection by% SC form retention of 64%.

4. Conclusion

Long chain aliphatic esters as well as organic and ferrocene containing Schiff bases were successfully synthesized. Results of different in vitro biocidal testing of Schiff bases and ferrocene Schiff bases exhibit that ferrocene Schiff bases had shown good antitumor (antimitotic), DPPH scavenging (antioxidant) and DNA



	P	X	Z1	Z1.1	A1	A2	A1.1	A2.1	AN1	AN2	AN 1.1	AN 2.1	FA1	FA2	FA1. 1	FA2. 1
Open circular %	19.3	0	52.1	39.8	43.2	48.7	38.1	39.6	64.9	53.5	69.4	53.5	58.4	45.4	45.6	35.8
Superoiled %	80.7	0	47.9	60.2	55.9	51.2	61.9	60.4	35.1	46.7	30.6	46.5	41.6	54.6	54.4	64.2

Fig. 1. DNA protective activity of synthesized organic compounds from oxidative damage. • All the synthesized organic compounds were tested at 10 μg/ml final concentration. • Plasmid pBR322 (P) was used as a DNA source mostly present in super-coiled form. • $FeSO_4$ and H_2O_2 generated OH radical by Fenton reaction in each test. • Total DNA damage was observed in case of positive control (X) where there was only the plasmid DNA in interaction with the Fenton reaction. • % super-coiled form of pBR322 DNA retained indicated protection while open-circular form represents damage in each treatment.

protecting activity as compared to other compound of same series. In this study a difference in biological activities was also noted between Schiff-base series and their reacting compounds. Considering significant antitumor and antioxidant activities of ferrocene Schiff bases they could be regarded as potential anticancer drug candidate with further advanced testing and optimization.

References

[1] B. Patwardhan, A.D.B. Vaidya, M. Chorghade, Curr. Sci. India 86 (2004) 789.
[2] M. MacCoss, T.A. Baillie, Science 303 (2004) 1810.
[3] K.E. Dombowski, W. Baldwin, J.E. Sheats, J. Organomet. Chem. 302 (1986) 281.
[4] R. Epton, G. Marr, G.K. Rogers, J. Organomet. Chem. 150 (1978) 93.
[5] P. Hu, K. Zhao, Hong-Bo Xu, Molecules (2001) M251.
[6] A. Togni, R.L. Halterman (Eds.), Metallocenes, Wiley-VCH Verlag GmbH, Weinheim, Germany, 1998.
[7] A. Togni, T. Hayashi (Eds.), Ferrocene: Homogenous Catalysis, Organic Synthesis, Material VCH, Wenheim, 1995.
[8] P. Stepnicka (Ed.), Ferrocenes: Ligands, Material and Biomolecules, John Wiley and Sons, 2008.
[9] O. Domarle, G. Blampain, H. Agnaniet, T. Nzadiyabi, J. Lebibi, J. Brocard, L. Maciejewski, C. Biot, A.J. Georges, P. Millet, Antimicrob. Agents Chemother. 42 (1998) 540.
[10] S. Top, A. Vessieres, C. Cabestaing, I. Laios, G. Leclecq, C. Provot, G. Jaouen, J. Organomet. Chem. 637 (2001) 500.
[11] S. Top, J. Tang, A. Vessieoes, D. Carrez, C. Provot, G. Jaouen, Chem. Commun. (1996) 955.
[12] C. Biot, G. Glorian, L. Maciejewski, J. Brocard, O. Domarle, G. Blampain, P. Millet, A.J. George, H. Abessolo, D. Dive, J. Lebibi, J. Med. Chem. 40 (1997) 3715.
[13] B. Halliwell, Ann. Rev. Nutr. 16 (1996) 33.
[14] V. Vrchovska, C. Sousa, P. Valenta, F. Ferreres, J.A. Pereira, Seabra, P.B. Andrade, Food. Chem. 98 (2006) 416.
[15] C. Behl, B. Moosmann, Free. Radic. Biol. Med. 33 (2002) 182.
[16] P.S. Coker, J. Radecke, C. Guy, N.D. Camper, Phytomedicine 10 (2003) 133.
[17] G.Y. Yeap, S.T. Ha, P.L. Lim, P.L. Boey, W.A.K. Mahmood, Mol. Cryst. 423 (2004) 73.
[18] G.Y. Yeap, S.T. Ha, P.L. Boey, W.A.K. Mahmood, Mol. Cryst. 452 (2006) 73.
[19] G.Y. Yeap, W.S. Ooi, Mol. Cryst. 381 (2002) 169.
[20] S. Sudhakar, T. Narasimhaswamy, K.S.V. Srinivasan, Liq. Cryst. 27 (2000) 1525.
[21] N.R. Ferrigni, J.E. Putnam, B. Anderson, L.B. Jacobsen, D.E. Nichols, D.S. Moore, J.L. McLaughlin, P.G. Powell, C.R.J. Smith, J. Nat. Prod. 45 (1982) 679.
[22] C.Y. Hsu, Biol. Res. 39 (2006) 281.
[23] Y.H. Tian, Food. Chem. 91 (2005) 413.
[24] D.L. Pavia, G.S. Lampman, G.S. Kriz, Introduction to Spectroscopy, Thomson Learning, 2001.
[25] A. Ullah, F.L. Ansari, I.U. Haq, S. Nazir, B. Mirza, Chem. Biodiv. (2007) 4203.
[26] S. Inaytullah, R. Irum, A.U. Rehman, M.F. Chaudhry, B. Mirza, Pharm. Biol. 45 (2007) 397.
[27] M.S. Ahmad, M. Hussain, M. Hanif, S. Ali, B. Mirza, Molecules 12 (2007) 2348.
[28] M. Hanif, M. Hussain, S. Ali, M.H. Bhatti, M.S. Ahmad, B. Mirza, H.S. Evans, Turk. J. Chem. 31 (2007) 349.
[29] M.S. Ahmad, M. Hussain, M. Hanif, S. Ali, B. Mirza, Chem. Biol. Drug Des, doi: 10.1111/j.1747-0285.2008.00668.x.
[30] A.C. Braun, Prog. Exp. Tumor. Res. 15 (1972) 165.
[31] A.G. Galsky, J.P. Wilsey, R.G. Powell, Plant Physiol. 65 (1980) 184.
[32] R. Kovjazin, T. Eldar, M. Patya, A. Vanichicin, H.M. Lander, A. Novogrodsky, FASEB J. 17 (2003) 467.
[33] G.H. Naik, K.I. Priyadarsini, J.G. Satav, M.M. Banavalikar, D.P. Sohoni, M.K. Biyani, H. Mohan, Phytochemistry 63 (2003) 97.
[34] X. Wu, E.R.T. Tiekink, L. Kostetski, N. Kocherginsky, A.L.C. Tan, S.B. Khoo, P. Wilairat, M. Go, Eur. J. Pharma. Sci. 27 (2006) 175.