# Synthesis, characterization, biological evaluation and QSAR of some Schiff base esters: Promising new antitumor, antioxidant and anti-inflammatory agents

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**Abstract**: We report the synthesis, characterization, biological evaluation and quantitative structure-activity relationship of some Schiff base esters as promising new antitumor, antioxidant and anti-inflammatory agents. The Schiff base esters were synthesized by two synthetic routes using variably substituted hydroxy benzaldehydes with para amino phenol in appreciable yields. All the newly synthesized esters have been characterized by $^{1}$H-NMR, $^{13}$C-NMR, FT-IR techniques and elemental analysis. The synthesized esters were examined for antioxidant, antitumor and anti-inflammatory potentials through different bioassays and quantitative structure-activity relationship was studied. Bioassays showed encouraging results and indicated that some of these title compounds may have potential for further pharmacological investigations.

**Keywords**: Schiff base esters, potato disc antitumor assay, free radical induced oxidative DNA damage analysis, Carrageenan induced rat hind paw edema test.

## INTRODUCTION

Schiff bases have been the subject of extensive interest due to versatility of their applications in various fields. Initially discovered by a synthetic chemist, Schiff bases soon became the research theme of other synthetic chemists, biologists, pharmacists and even the physicists. Schiff bases can be represented by the general formula $(R_1 R_2)C=N-R_3$, where $R_3$ group is a alkyl or phenyl which makes the Schiff base a very stable imine. Schiff bases can be prepared from a carbonyl compound such as an aldehyde or a ketone and an amine by a nucleophilic addition reaction resulting in a hemiaminal, which generates an imine after dehydration (Jarrahpour and Zarei, 2004).

Schiff bases are very important compounds in pharmaceutics and medicines (Yusuf *et al*., 2007). They show a range of biological activities such as herbicidal, antibacterial, antiviral, antifungal, anti-inflammatory and antioxidant activities (Karatepe and Karatas, 2006; Wang *et al*., 1990; Pattanashetti *et al*., 1984; Iqbal *et al*., 2005; Tai *et al*., 1984; Tang *et al*., 1985).

In addition Schiff bases are also used in the synthesis of compounds with bioactivity such as β-lactams (Jarrahpour and Zarei, 2004). Metal complexes of Schiff bases are known to posses antifungal, antimalarial, antineoplastic, antibacterial and anti-cancerous effects (Oner *et al*., 2005; Al-Allaf *et al*., 1996). Cytotoxic and anti-proliferative effects of some Schiff bases on A549 (human small lung cancer) and HeLa (human cervix cancer) cell lines have also been reported (Yusuf *et al*., 2007). Nitro and halo derivatives of Schiff bases have antimicrobial and antitumor activities (Das *et al*., 1996). Furthermore, Schiff bases possess a variety of interesting results including inhibition of anti-mosquito larvae, herpes simplex (HSV-1) virus type 1, adenovirus and anti-mouse hepatitis virus (MHV) (Das *et al*., 1996).

Schiff base polymers have also been reported to show superior mechanical, thermal, electrical and dielectric properties (Giamberini *et al*., 1995; Zongyong *et al*., 2007). Last but not the least, the liquid crystalline properties of Schiff base derivatives have also made them attractive as technologically important materials (Zongyong *et al*., 2007).

In the recent years, ester derivatives of aromatic Schiff bases have been reported to have liquid crystalline properties (Yeap *et al*., 2004; 2006). Previously we have reported the synthesis, characterization of some new Schiff base esters and their liquid crystalline properties. Due to immense pharmacological significance of the Schiff bases and their derivatives we planned to synthesize, characterize and biologically evaluate these derivatives as promising new antitumor, antioxidant and anti-inflammatory agents. Here we report, modified Schiff bases with aliphatic and aromatic side chains and their antitumor, antioxidant and anti-inflammatory activities with quantitative structure-activity relationship. The chemical structures were established using elemental

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analysis, FT-IR) spectroscopy, mass spectrometry and Nuclear Magnetic Resonance (NMR) spectroscopy. These compounds were then tested for their potential as antitumor, antioxidant and anti-inflamatory agents.

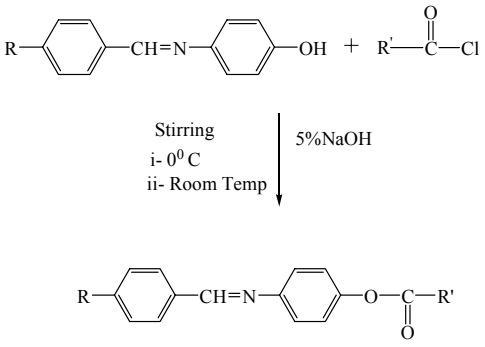
## MATERIALS AND METHODS

### *General*

Melting points were checked on apparatus Electrothermal 9100 melting point. Spectra IR were recorded on a FTS 3000 MX, Bio- Rad Merlin Fourier Transform Infra Red spectrophotometer. Solid samples were taken in KBr pellets for recording their spectra. Elemental analysis was carried out on CHNS932 LECO elemental analyzer. $^{1}$H NMR & $^{13}$C NMR spectra were recorded at 300 MHz using a Bruker AV spectrometer and Tetramethylsilane (TMS) was used as internal standard. Chemical shift values (δ) were mentioned in ppm. The optical rotation of the compounds was taken with ATAGO, AP-100 automatic polarimeter. All chemicals were obtained from either Merck or Aldrich. The solvents were purified before use and dried by standard methods (Furniss *et al*., 1989).

### *Synthesis*

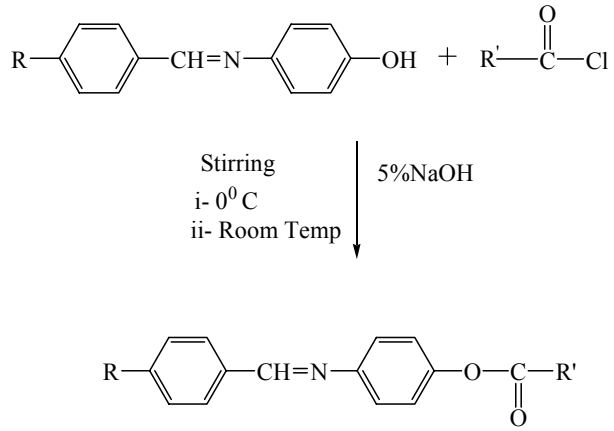
The Schiff bases were synthesized by heating at reflux an equimolar quantity of 4-aminophenol (20 mmol) and aldehyde (20 mmol) in ethanol for 5 hours (scheme 1). The resulting products were recrystallized from ethanol. The Schiff base esters were synthesized by two different synthetic strategies.




**Scheme 1**: General Synthetic route of Schiff bases.

### *Synthetic route for compounds 1-5*

Compounds **1-5** were synthesized by esterification using thionyl chloride (scheme 2) (Popp and Roth, 1963). Acid chlorides were synthesized by heating at reflux with ratios of thionyl chloride (12mmol) and corresponding carboxylic acids (10 mmol). After the completion of reaction, under-reduced pressure the excess of thionyl chloride was removed. Schiff base (0.5 g) was stirred with 5 ml of 5% sodium hydroxide solution at room temperature to get transparent solution then it was chilled and carboxylic acid chloride was added to it stepwise with constant stirring at 0°C for one hour. Then the mixture was stirred for three hours at room temperature. Solid product was filtered and recrystallized from ethanol.




**Scheme 2**: General synthetic route of Schiff base esters (compounds **1-5**).

### *N-benzylidene-4-lauroyloxyaniline 1*

Compound **1** was prepared by using thionyl chloride (1.428 g, 12 mmol), lauric acid (2 g, 10mmol) and N-benzylidene-4-hydroxyaniline (0.5 g). Yield 66%; mp 123°C. $^{1}$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H, C*H*=N), 7.93 (d, *J*=3.4 Hz, 2H, *H*-C(2′,6′)), 7.51-7.47 (m, 3H, *H*-C(3′,4′,5′)), 7.25 (d, *J*=8.7 Hz, 2H, *H*-C(2,6)), 7.13 (d, *J*=8.7 Hz, 2H, *H*-C(3,5)), 2.57 (t, *J*=7.2 Hz, 2H, C$H_2$-C=O), 1.78 (qn, *J*=6.9 Hz, 2H, O=C-CH$_2$-C$H_2$-CH$_2$), 1.28 (m, 16H, (C$H_2$)$_8$), 0.90 (t, *J*=6.9 Hz, 3H, C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 172.5, 160.5, 149.5, 148.9, 136.6, 131.4, 129.1, 122.7, 121.7, 115.6, 34.4, 31.8, 29.7, 29.6, 29.50, 29.39, 29.30, 29.15, 24.9, 22.7, 14.1. IR (KBr) ν (cm$^{-1}$): 1753(C=O), 1624(C=N), 1598(C=C). Anal. Calcd. for $C_{25}H_{33}NO_2$ :C, 79.11; H, 8.76; N,3.69, Found: C, 78.97; H, 8.92; N,3.53.

### *N-benzylidene-4-myristoyloxyaniline 2*

Compound **2** was prepared similarly as compound **1** using thionyl chloride (1.428 g, 12 mmol), myristic acid (2.28 g, 10mmol) and N-benzylidene-4-hydroxyaniline (0.5 g).Yield 58%; m.p. 80°C. $^{1}$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H, C*H*=N), 7.91 (d, *J*=3.4 Hz, 2H, *H*-C(2′,6′)), 7.56-7.48 (m, 3H, *H*-C(3′,4′,5′)), 7.25 (d, *J*=8.7 Hz, 2H, *H*-C(2,6)), 7.13 (d, *J*=8.7 Hz, 2H, *H*-C(3,5)), 2.57 (t, *J*=7.2 Hz, 2H, C$H_2$-C=O), 1.78 (qn, *J*=6.9 Hz, 2H, O=C-CH$_2$-C$H_2$-CH$_2$), 1.28 (m, 20H, (C$H_2$)$_{10}$), 0.90 (t, *J*=6.9 Hz, 3H, C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 172.5, 160.5, 149.5, 148.9, 136.6, 131.4, 129.1, 122.7, 121.7, 115.6, 34.4, 33.6, 31.8, 29.7, 29.6, 29.50, 29.39, 29.30, 29.15, 24.9, 22.7, 14.1. IR (KBr) ν (cm$^{-1}$): 1747(C=O), 1622(C=N), 1593(C=C). Anal. Calcd. for $C_{27}H_{37}NO_2$: C, 79.56; H, 9.15; N, 3.44, Found: C, 79.21; H, 8.97; N,3.21.

### *N-benzylidene-4-(4″-methyl)benzoyloxyaniline 3*

Compound **3** was prepared similarly as compound **1** using thionyl chloride (1.428 g, 12 mmol), p-methylbenzoic

acid (1.36 g, 10mmol) and N-benzylidene-4-hydroxyaniline (0.5 g).Yield 57%; m.p. 247°C. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.46 (s, 1H, C*H*=N), 8.12 (d, *J*=8.4 Hz, 2H, *H*-C(2″,6″)), 7.90 (d, *J*=8.7 Hz, 2H, *H*-C(2′,6′)), 7.35-7.21 (m, 7H, Ar), 7.12 (d, *J*=9 Hz, 2H, *H*-C(3,5)), 2.49 (s, 3H, Ar-C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.4, 158.9, 149.2, 144.5, 137.5, 134.6, 130.3, 130.0, 129.4, 129.1, 128.2, 126.7, 122.5, 121.9, 21.5. IR (KBr) ν ($cm^{-1}$): 1727(C=O), 1626(C=N), 1604(C=C). Anal. Calcd. for $C_{21}H_{17}NO_2$: C, 79.98; H, 5.43; N,4.44, Found: C, 79.51; H, 5.21; N,4.28.

***N-benzylidene-4-[2″-{4‴-(2⁗-methyl propyl) phenyl}] propanoyloxyaniline 4***

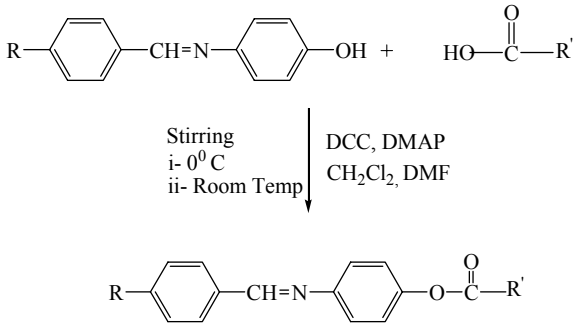
Compound **4** was prepared similarly as compound **1** using thionyl chloride (1.428 g, 12 mmol), ibuprofen (2.06 g, 10mmol) and N-benzylidene-4-hydroxyaniline (0.5 g) .Yield 72%; m.p. 116°C. $[\alpha]_D^{22.7}$ = +4.89° (c=20 mg/20ml).$^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H, C*H*=N ), 7.38-6.86 (m, 13H, Ar), 3.92 (q, *J*=7.2 Hz, 1H, O=C-C*H*), 3.67 (q, *J*=7.2 Hz, 1H, O=C-C*H*), 2.48 (d, *J*=7.2 Hz, 2H, C$H_2$-Ar), 1.87 (m, 1H, C*H*), 1.58 (m, 3H, C$H_3$), 0.92 (d, *J*=6.6 Hz, 6H, 2C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 173.4, 172.4, 146.8, 141.2, 140.8, 137.9, 137.1, 135.5, 129.9, 129.5, 127.4, 127.2, 121.7, 120.4, 47.6, 45.0, 30.2, 22.4, 18.58, 18.51. IR (KBr) ν ($cm^{-1}$): 1724(C=O), 1638(C=N), 1605(C=C). Anal. Calcd. for $C_{26}H_{27}NO_2$: C, 81.01; H, 7.06; N,3.63, Found: C, 80.9; H, 7.2; N, 3.35.

***N-4′-methoxybenzylidene-4-[2″-{4‴-(2⁗-methylpropyl)phenyl} propanoyloxyaniline 5***

Compound **5** was prepared similarly as compound **1** using thionyl chloride (1.428 g, 12 mmol), ibuprofen (2.06g, 10mmol) and N-4′-methoxybenzylidene-4-hydroxyaniline (0.5 g). Yield 72%; m.p. 108°C. $[\alpha]_D^{22.7}$ = +0.91° (c=20 mg/20ml). $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H, C*H*=N ), 7.38-6.97 (m, 12H, Ar), 3.92 (q, *J*=7.2 Hz, 1H, O=C-C*H*), 3.86 (s, 3H, O-C$H_3$) 3.67 (q, *J*=7.2 Hz, 1H, O=C-C*H*), 2.48 (d, *J*=7.2 Hz, 2H, C$H_2$-Ar), 1.87 (m, 1H, C*H*), 1.58 (m, 3H, C$H_3$), 0.92 (d, *J*=6.6 Hz, 6H, 2C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 173.4, 172.4, 153.8, 141.2, 140.8, 137.9, 137.1, 135.5, 129.9, 129.5, 127.4, 127.2, 121.7, 120.4, 55.7, 47.6, 45.0, 30.2, 22.4, 18.58, 18.51. IR (KBr) ν ($cm^{-1}$): 1740(C=O), 1627(C=N), 1600(C=C). Anal. Calcd. for $C_{27}H_{29}NO_3$: C, 78.04; H, 7.03; N,3.37, Found: C, 77.95; H, 6.98; N, 3.16.

***Synthetic procedure for compounds 6-14***

Schiff bases were esterified to compounds **6-14** by variably substituted carboxylic acids and DMAP in DMF: DCM mixture at 0°C (scheme 3) (Sudhakar *et al*., 2000). Schiff base (3.8 mmol), carboxylic acid (7.6 mmol) and DMAP (0.76 mmol) were dissolved in 10 ml of dry DCM: DMF mixture (50:50) and stirred at 0°C., DCC (7.6 mmol) dissolved in 10 ml of DCM was added drop wise to this solution and stirred at 0°C for 1 hr. The solution was stirred at room temperature for 12 hrs. Finally, the reaction mixture was filtered and DCM was removed from the filtrate by evaporation and the DMF solution was precipitated in water. These precipitates were filtered, washed with water and methanol, and then recrystallized from ethanol.




**Scheme 3**: General synthetic route of Schiff base esters (compounds **6-14**).

***N-benzylidene-4-(3″-phenyl)butyroyloxyaniline 6***

Compound **6** was prepared by using N-benzylidene-4-hydroxyaniline (0.75 g, 3.8 mmol) and 3-phenylbutyric acid (1.25 g, 7.6 mmol). Yield 61%; m.p. 97°C. $[\alpha]_D^{22.7}$ = -0.73° (c=20 mg/20ml). $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H, C*H*=N), 7.49 (d, *J*=8.4 Hz, 2H, *H*-C(2′,6′)), 7.39-7.20 (m, 8H, Ar), 7.16 (d, *J*=9 Hz, 2H, *H*-C(2,6)), 7.12 (d, *J*=9 Hz, 2H, *H*-C(3,5)), 3.43 (sx, *J*=7.2 Hz, 1H, C-*H*), 2.93-2.79 (m, 2H, C$H_2$), 1.44 (d, *J*=6.9 Hz, 3H, C$H_3$), . $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.1, 162.3, 159.8, 149.9, 148.4, 145.2, 130.5, 129.1, 128.6, 126.9, 126.6, 122.1, 121.6, 114.2, 43.1, 36.8, 21.9. IR (KBr) ν ($cm^{-1}$): 1745(C=O), 1628(C=N), 1598(C=C). Anal. Calcd. for $C_{23}H_{21}NO_2$: C, 80.44; H, 6.16; N,4.08, Found: C, 79.94; H, 6.09; N, 3.96.

***N-benzylidene-4-naphthoyloxyaniline 7***

Compound **7** was prepared similarly as compound **6** using N-benzylidene-4-hydroxyaniline (0.75 g, 3.8 mmol) and 1-naphthalenecarboxylic acid (1.30 g, 7.6 mmol).Yield 60%; m.p. 185ºC. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H, C*H*=N), 9.09-7.24 (m, 14H, Ar), 7.10 (d, *J*=8.7 Hz, 2H, *H*-C(3,5)). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.4, 162.7, 157.0, 149.8, 145.1, 134.9, 133.9, 133.2, 131.3 130.4, 130.2, 128.8, 128.3, 126.7, 126.3, 126.1, 124.6, 123.7, 117.8, 115.4. IR (KBr) ν ($cm^{-1}$): 1728(C=O), 1622(C=N), 1607(C=C). Anal. Calcd. for $C_{24}H_{17}NO_2$: C, 82.03; H, 4.88; N,3.99, Found: C, 81.9; H, 4.54; N, 3.47.

***N-benzylidene-4-cinnamoyloxyaniline 8***

Compound **8** was prepared similarly as compound **6** using N-benzylidene-4-hydroxyaniline (0.75 g, 3.8 mmol) and cinnamic acid (1.12g, 7.6mmol). Yield 68%; m.p. 128ºC. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.45 (s, 1H,

C*H*=N), 7.9-7.1 (m, 14H, Ar), 7.49 (d, *J*=8.3 Hz, 1H, *H*-C=CH), 7.42 (d, *J*=8.1 Hz, 1H, HC=C-*H*) . $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 179, 165, 153, 150, 147, 143, 138, 136, 132, 130, 129, 125, 120, 118, 112. IR (KBr) ν ($cm^{-1}$): 1728(C=O), 1622(C=N), 1607(C=C). Anal. Anal. Calcd. for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N,4.28, Found: C, 81.9; H, 5.06; N, 4.17.

***N-4′-methoxybenzylidene-4-(3″-phenyl)butyroyloxyaniline 9***

Compound **9** was prepared similarly as compound 6 using N-4′-methoxybenzylidene-4-hydroxyaniline (0.86 g, 3.8 mmol) and 3-phenylbutyric acid (1.25 g, 7.6 mmol). Yield 54%; m.p. 103ºC. $[\alpha]_D^{22.7}$ = +0.27° (c=20 mg/20ml). $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.37 (s, 1H, C*H*=N), 7.85 (d, *J*=8.7 Hz, 2H, *H*-C(2′,6′)), 7.39-7.25 (m, 5H, Ar), 7.16 (d, *J*=9 Hz, 2H, *H*-C(2,6)), 7.00 (d, *J*=9 Hz, 2H, *H*-C(3,5)), 6.93 (d, *J*=9 Hz, 2H, *H*-C(3′,5′)), 3.89 (s, 3H, O-C$H_3$), 3.43 (sx, *J*=7.2 Hz, 1H, C-*H*), 2.93-2.79 (m, 2H, C$H_2$), 1.43 (d, *J*=6.9 Hz, 3H, C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.1, 162.3, 159.8, 149.9, 148.4, 145.2, 130.5, 129.1, 128.6, 126.9, 126.6, 122.1, 121.6, 114.2, 55.5, 43.1, 36.8, 21.9. IR (KBr) ν ($cm^{-1}$): 1737(C=O), 1622(C=N), 1597(C=C). Anal. Calcd. for $C_{24}H_{23}NO_3$: C, 77.19; H, 6.21; N,3.75, Found: C, 76.54; H, 6.01; N, 3.38.

***N-4′-methoxybenzylidene-4-naphthoyloxyaniline 10***

Compound **10** was prepared similarly as compound **6** by using N-4′-methoxybenzylidene-4-hydroxyaniline (0.86 g, 3.8 mmol) and 1-naphthalenecarboxylic acid (1.30 g, 7.6 mmol). Yield 62%; m.p. 101ºC. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.43 (s, 1H, C*H*=N), 9.09-7.28 (m, 11H, Ar), 7.10 (d, *J*=8.7 Hz, 2H, *H*-C(3,5)), 6.79 (d, *J*=8.7 Hz, 2H, *H*-C(3′,5′)), 3.95 (s, 3H, O-C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.4, 162.7, 159.2, 157.0, 149.8, 145.1 134.9, 133.9,133.2, 130.4, 130.2, 128.8, 128.3, 126.7, 126.3, 126.1, 124.6, 123.7, 117.8, 115.4, 55.3. IR (KBr) ν ($cm^{-1}$): 1729(C=O), 1630(C=N), 1604(C=C). Anal.Calcd. for $C_{25}H_{19}NO_3$: C, 78.72; H, 5.02; N,3.67, Found: C, 77.91; H, 4.98; N, 3.15.

***N-4′-chlorobenzylidene-4-lauroyloxyaniline 11***

Compound **11** was prepared similarly as compound **6** the same procedure using N-4′-chlorobenzylidene-4-hydroxyaniline (0.88 g, 3.8 mmol) and lauric acid (1.52 g, 7.6 mmol). Yield 70%; m.p. 91-102ºC. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.43 (s, 1H, C*H*=N), 7.85 (d, *J*=9 Hz, 2H, *H*-C(2′,6′)), 7.45 (d, *J*=8.4 Hz, 2H, *H*-C(3′,5′)), 7.24 (d, *J*=8.7 Hz, 2H, *H*-C(2,6)), 7.12 (d, *J*=8.7 Hz, 2H, *H*-C(3,5)), 2.58 (t, *J*=7.5 Hz, 2H, C$H_2$-C=O), 1.76 (qn, *J*=7.2 Hz, 2H, O=C-$CH_2$-C$H_2$-$CH_2$), 1.29 (m, 16H, (C$H_2$)$_8$), 0.90 (t, *J*=7.2 Hz, 3H, C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 172.5, 159.8, 149.8, 138.9, 133.2, 131.5, 130.6, 129.1, 122.2, 121.7, 34.4, 31.8, 29.7, 29.6, 29.50, 29.39, 29.30, 29.15, 24.9, 22.7, 14.3. IR (KBr) ν ($cm^{-1}$): 1748(C=O), 1626(C=N), 1595(C=C). Anal.Calcd. for $C_{25}H_{32}NO_2Cl$: C, 72.53; H, 7.79; N,3.38, Found: C, 71.72; H, 7.38; N, 3.04.

***N-4′-chlorobenzylidene-4-naphthoyloxyaniline 12***

Compound **12** was prepared similarly as compound **6** using N-4′-chlorobenzylidene-4-hydroxyaniline (0.88 g, 3.8 mmol) and 1-naphthalenecarboxylic acid (1.30 g, 7.6 mmol). Yield 69%; m.p. 122ºC. $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.43 (s, 1H, C*H*=N), 9.09-7.33 (m, 11H, Ar), 7.24-7.10 (m, 4H, Ar). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.4, 162.7, 157.0, 154.5, 149.8, 145.1 134.9, 133.9,133.2, 130.4, 130.2, 128.8, 128.3, 126.7, 126.3, 126.1, 124.6, 123.7, 117.8, 115.8. IR (KBr) ν ($cm^{-1}$): 1738(C=O), 1621(C=N), 1601(C=C). Anal.Calcd. for $C_{24}H_{16}NO_2Cl$: C, 74.71; H, 4.18; N,3.63, Found: C, 73.65; H, 3.97; N, 3.03.

***N-4′-methylbenzylidene-4-(3″-methyl)benzoyloxyaniline 13***

Compound **13** was prepared similarly as compound **6** using N-4′-methylbenzylidene-4-hydroxyaniline (0.80 g, 3.8 mmol) and *m*-methylbenzoic acid (1.03 g, 7.6 mmol). Yield 65%; m.p. 168-250ºC. $^1$H NMR ( 300 MHz, $CDCl_3$) δ (ppm): 8.45 (s, 1H, C*H*=N), 8.11 (d, *J*=8.7 Hz, 2H, *H*-C(2″,6″)), 7.83 (d, *J*=8.1 Hz, 2H, *H*-C(2′,6′)), 7.31-7.22 (m, 8H, Ar), 2.48 (s, 3H, Ar-C$H_3$), 2.45 (s, 3H, Ar-C$H_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.4, 158.9, 149.3, 149.2, 144.5, 137.5, 134.6, 130.3, 130.0, 129.4, 129.1, 126.7, 122.5, 121.9, 21.8, 21.4. IR (KBr) ν ($cm^{-1}$): 1742(C=O), 1626(C=N), 1601(C=C). Anal.Calcd. for $C_{22}H_{19}NO_2$: C, 80.22; H, 5.81; N,4.25, Found: C, 79.93; H, 5.34; N, 4.01.

***N-4′-methylbenzylidene-4-(3″-phenyl)butyroyloxyaniline 14***

Compound **14** was prepared similarly as compound **6** using N-4′-methylbenzylidene-4-hydroxyaniline (0.80 g, 3.8 mmol) and 3-phenylbutyric acid (1.25 g, 7.6 mmol). Yield 52%; m.p. 106ºC. $[\alpha]_D^{22.7}$ =+1.69° (c=20 mg/20ml). $^1$H NMR( 300 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H, C*H*=N), 7.85 (d, *J*=8.7 Hz, 2H, *H*-C(2′,6′)), 7.37-7.25 (m, 5H, Ar), 7.16 (d, *J*=9 Hz, 2H, *H*-C(2,6)), 7.12 (d, *J*=9 Hz, 2H, *H*-C(3,5)), 7.01 (d, *J*=9 Hz, 2H, *H*-C(3′,5′)), 3.43 (sx, *J*=7.2 Hz, 1H, C-*H*), 2.93-2.79 (m, 2H, C$H_2$), 1.44 (d, *J*=6.9 Hz, 3H, C$H_3$), . $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.1, 162.3, 159.8, 149.9, 148.4, 145.2, 130.5, 129.1, 128.6, 126.9, 126.6, 122.1, 121.6, 114.2, 43.1, 36.8, 23.9, 21.9. IR (KBr) ν ($cm^{-1}$): 1739(C=O), 1623(C=N), 1593(C=C). Anal.Calcd. for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N,3.92, Found: C, 79.79; H, 5.98; N, 3.63.

## BIOLOGICAL ASSAYS

***Potato disc antitumor assay***

Potato disc anti-tumor assay as reported earlier was used *in vitro* to detect the antitumor potential of newly

synthesized Schiff base esters (Rehman *et al*., 2001; Hanif *et al*., 2007; Ansari *et al*., 2008; Nawaz *et al*., 2009). In this assay, culture of *Agrobacterium tumefaciens* (At 10) strain (48 hrs old) was used as agent to form tumors on discs of potatoes. Each test compound was checked for their tumor inhibitory potential at all concentrations i.e. 10, 100, and 1000 µg/ml with vincristine as positive control and THF used as negative control. Under sterilized conditions, potato discs (0.5 cm thickness) were made by using sterilized instruments from surface sterilized ($HgCl_2$ 0.1%) red healthy potatoes. Ten discs of potato were transferred on each petriplate with 1.5% agar medium for support. These petriplates were incubated for 21 days at 28°C for tumor induction after treatment with test compounds and At 10 strain on each disc. Number of tumors was counted after staining with the help of dissecting microscope. Lugol's solution (5% $I_2$ and 10% KI) was used for staining. Percentage tumor inhibition was calculated. Formula for calculation of percentage tumor inhibition is given in table 2. Each experiment was performed in triplicate and $IC_{50}$ values for each compound were also calculated (table 2).

***Free radical induced oxidative DNA damage assay***

Free radical induced oxidative DNA damage assay was carried out *in vitro* to study the protection or damage of DNA qualitatively by test samples (Schiff base esters) *in vitro* as reported earlier (Hsu, 2006; Tian and Hua, 2005; Zaheer *et al*., 2010). Phosphate buffer (50 mM) pH 7.4 was used to dilute Plasmid pBR322 DNA (Fermentas, Germany) to get a concentration of 0.5 µg/3 µl. Reaction mixture (a total volume of 15 µl kept in eppendorf tube) contained diluted pBR322 (0.5 µg/3 µl of 50 mM phosphate buffer), 5 µl of test compounds at various concentrations (10, 100 and 1000 µg/ml), 3 µl of 2 mM $FeSO_4$ and 4 µl of 30% $H_2O_2$. In each experiment a 1 Kb DNA marker (L), plasmid pBR322 DNA (P) as positive control and plasmid pBR322 DNA treated with $H_2O_2$ and $FeSO_4$ (Fenton reagent, X) as negative control were also used. To avoid photoexcitation of test compounds, all the reaction mixtures were incubated at 37ºC in an incubator for 1 h in dark. All reaction mixtures were subjected to 1% agarose gel electrophoresis in 1X TBE buffer. DNA bands (supercoiled, open circular and linear) were stained with ethidium bromide dye. Gels were documented by using instrument Gel Doc (Bio Rad) and band intensity was determined.

***Carrageenan induced edema test in rat hind paw***

Carrageenan induced edema in rat hind paw is prescreen or standard experiment of acute inflammation and is one of the most widely used tests for the screening of new agents which can prove anti-inflammatory activity (Shaheen *et al*., 2008; Chan *et al*., 1995). Carrageenan is used for testing of anti-inflammatory agents and it does not have any antigenic effects and it is also devoid of apparent systemic effects (Winter *et al*., 1962). Anti-inflammatory potential of all the test samples (Schiff base esters) was carried out by carrageenan induced edema test in rat paw in albino rats (180-220 Kg body weight) of either sex, according to the previously reported method, using three rats in each group (Shaheen *et al*., 2008). The experimental animals (rats) were given carrageenan (1% w/v suspension in 0.9% saline) 0.05ml under the planter aponeurosis area in the right rat hind paw. One hour before carrageenan injection, the test animals groups were given 25 $mgkg^{-1}$ dose of test compounds (Schiff base esters) orally suspended in 0.75% CMC Naproxen 3467 Sodium (5 $mlkg^{-1}$ body weight). The control animals were given the dose in volume (5 $mlkg^{-1}$ body weight) of 0.75% CMC Sodium as in the test group animals. 10 $mgkg^{-1}$ of Diclofenac Potassium suspended in the 0.75% CMC Sodium (5 $mlkg^{-1}$ body weight) as standard drug was given orally to another group of rats, 1 h before carrageenan injection. The edema was measured at 0, 1, 2, 3, and 4 hour after carrageenan injection by using plethysmometer (Ugo Basile). Experimental model animals (albino rats) were kept under observation for one month after completion of experiment and compared with the control group. For each group, the percent inhibition of edema was calculated with respect to control group by using the relation. Percentage edema inhibition was calculated with the help of formula given in table 3.

## RESULTS

***Synthesis and characterization***

The exact procedure/route followed for the synthesis of the Schiff bases and their corresponding esters are presented in schemes 1, 2 and 3 (Mishra and Krishna, 2006; Gopalkrishnan *et al*., 2007). The Shiff bases containing OH and acid chlorides of different acids, in NaOH presence, gave moderate to good yield of compounds **1**-**5** (Scheme 2). The ester derivatives of Schiff bases **6**-**14** were synthesized by condensing the N-R-benzylidene-4-hydroxyanilines with different carboxylic acids using DCC and DMAP (a proton transfer catalyst) in DCM/DMF solvent mixture, in moderate to good yield (Scheme 3). All the esters were obtained as solids and were recrystallised using ethanol. All the compounds except **11** and **13** gave sharp melting points. The later two compounds gave a range of melting points with milky appearance during melting. The purity of the Schiff base esters was evaluated by TLC in n-hexane and acetone ratio (2:1 and 3:1) and confirmed by elemental analysis, which are in agreement with the calculated values. List of synthesized compounds is given in table 1.

***Infrared spectroscopy***

The Schiff bases esters were characterized by FTIR spectroscopy. The conversion of hydroxyl-substituted Schiff bases to their ester derivatives was confirmed by the disappearance of a broad absorption band of OH group around 3360-3328 $cm^{-1}$ and appearance of a strong

absorption band around 1757-1724 $cm^{-1}$ due to the presence of a carbonyl group of ester.

***NMR spectroscopy***

All the newly synthesized test compounds were also characterized by using $^{1}H$ and $^{13}C$ NMR spectroscopy.

**$^{1}$H-NMR**

Characteristic signals for the phenolic and azomethine protons appeared downfield as singlets around 9.63-9.52 ppm (O-H) and 8.67-8.56 ppm (CH=N) respectively, in addition to the aromatic protons which were observed between 7.90-6.73 ppm (Sannier and Siddiqi, 2001). In the case of methoxy substituted Schiff bases a singlet at 3.82 ppm was assigned to -$OCH_3$. The $^{1}$H-NMR spectra of all the Schiff base esters also confirmed the complete conversion of hydroxyl substituted Schiff bases to their corresponding esters by the disappearance of the downfield singlet for the phenolic proton, while the signals for the azomethine and aromatic protons appeared at their respective positions with slight difference. The $^{1}$H NMR spectra of the esters derived from aliphatic acids had almost the same pattern with slight differences due to different substitutions at the 4′-positions, while the difference in the length of alkyl chain had no marked effect on NMR signals.

**$^{13}$C-NMR**

The $^{13}$C-NMR spectra of the Schiff base esters showed characteristic downfield signals for the carbonyl and azomethine carbons around 173-165 ppm and 160-159 ppm. The aromatic carbons were observed between 159-114 ppm, while in the case of esters derived from aliphatic acids a number of signals were obtained in the aliphatic region.

## BIOLOGICAL EVALUATION

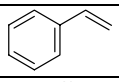
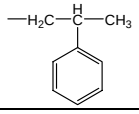
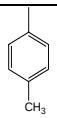
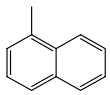
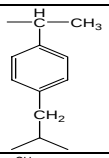
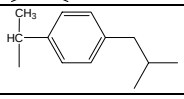
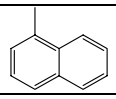
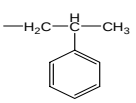
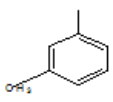
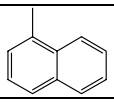
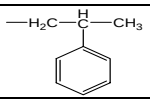
***Potato disc antitumor assay***

Potato disc antitumor assay is a reliable mean to detect potential antitumor agents (Ferrigni *et al*., 1982; Zaheer *et al*., 2010). All the synthesized Schiff base esters (compounds **1-14**) were investigated against *Agrobacterium tumefaciens* (At 10) induced tumors in potato discs for their antitumor potential at different concentrations (10, 100 and 1000 µg/ml) of synthesized compounds. Results are summarized in table 2.

***Free radical induced oxidative DNA damage assay***

Anti-oxidant and pro-oxidant effects of the synthesized Schiff base esters were investigated *in vitro* qualitatively by using free radical induced oxidative DNA damage assay at various concentrations (10, 100 and 1000 µg/ml) (Zaheer *et al*., 2010; Tian and Hua, 2005). In the present

**Table 1**: List of the title compounds

| Compd. | R | R' | Compd. | R | R' |
|---|---|---|---|---|---|
| **1** | H | -$(CH_2)_{10}CH_3$ | **8** | H |  |
| **2** | H | -$(CH_2)_{12}CH_3$ | **9** | $OCH_3$ |  |
| **3** | H |  | **10** | $OCH_3$ |  |
| **4** | H |  | **11** | Cl | -$(CH_2)_{10}CH_3$ |
| **5** | $OCH_3$ |  | **12** | Cl |  |
| **6** | H |  | **13** | $CH_3$ |  |
| **7** | H |  | **14** | $CH_3$ |  |

study, it was found that all the synthesized compounds showed plasmid DNA protection (antioxidant effect) at different concentrations (fig. 1). Moreover it was noted that the compounds showed antioxidant effect on DNA in dose dependent manner i.e. antioxidant effect on DNA increased with increase in concentration of the compound.

***Carrageenan induced edema test in rat hind paw***

In the present study, this test was used to determine anti-inflammatory effect of the newly synthesized Schiff base esters *in vivo* on carrageenan induced edema in rat hind paw (Yaqub *et al*., 2009). In the early phase of inflammation, i.e. at first hour, four compounds showed more than 50% anti-inflammatory potential ranging from 51.42 to 88.86% (compound **5**, **6**, **7** and **9**). Compound **5**

**Table 2**: Anti-tumor activities of synthesized compounds (Schiff base esters) [A]

| Compd. | % inhibition of tumors[B,C] | | | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| | 10 (µg/ml) | 100 (µg/ml) | 1000 µg/ml) | |
| **1** | 49.36 | 55.44 | 69.70 | 14.47 |
| **2** | 10.42 | 68.75 | 72.87 | 36.20 |
| **3** | 29.28 | 40.77 | 66.93 | 297.02 |
| **4** | 46.85 | 66.40 | 78.98 | 13.42 |
| **5** | 47.76 | 59.34 | 65.30 | 13.57 |
| **6** | 47.44 | 89.30 | 91.54 | 10.77 |
| **7** | 55.48 | 60.56 | 88.97 | 8.03 |
| **8** | 17.42 | 43.80 | 63.50 | 170.46 |
| **9** | 69.30 | 80.00 | 90.72 | 0.157 |
| **10** | 48.22 | 59.34 | 76.90 | 15.40 |
| **11** | 43.14 | 56.28 | 70.68 | 34.75 |
| **12** | 37.76 | 49.28 | 62.40 | 113.92 |
| **13** | 24.77 | 41.64 | 77.78 | 204.08 |
| **14** | 46.16 | 61.77 | 83.48 | 18.98 |
| **Vin*** | 100 | 100 | 100 | 0.003 |
| **THF** | - | - | - | - |

[A]Potato disc antitumor assay, More than 20% inhibition is significant. [B]Percentage tumor inhibition = (1- NS/NC) *100
Where NS = Average no of tumors in sample and NC = Average no of tumors in negative control.
[C]Values represents average percentage tumor inhibition of three replicates. *Vincristine (Reference drug)

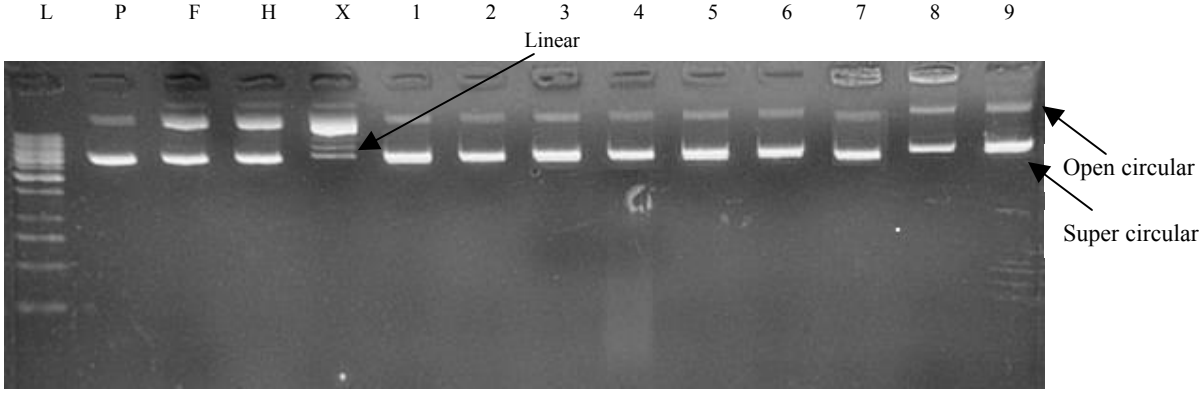



**Fig. 1**: Effect of compound **5**, **9** and **13** on pBR322 plasmid DNA.

| | | | |
|---|---|---|---|
| **L** | 1 KB marker/ladder | **L** | 1 KB marker/ladder |
| **P** | plasmid pBR322 | **F** | plasmid pBR322 treated with FeSO4 |
| **H** | plasmid pBR322 treated with H2O2 | | |
| **X** | (negative control) plasmid pBR322 treated with H2O2 and FeSO4 | | |
| **Lane 1** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **5** (10 µg/ml) | | |
| **Lane 2** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **5** (100 µg/ml) | | |
| **Lane 3** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **5** (1000 µg/ml) | | |
| **Lane 4** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **9** (10 µg/ml) | | |
| **Lane 5** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **9** (100 µg/ml) | | |
| **Lane 6** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **9** (1000 µg/ml) | | |
| **Lane 7** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **13** (10 µg/ml) | | |
| **Lane 8** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **13** (100 µg/ml) | | |
| **Lane 9** | plasmid pBR322 treated with H2O2 and FeSO4 + compound **13** (1000µg/ml) | | |

was found to be the most active compound in the early phases of inflammation with 88.86 % anti-inflammatory activity. In the late phase of inflammation, i.e. at 4th hour six of the fourteen test compounds (compound **6**, **7**, **9**, **10**, **12** and **13**) showed more than 70 % anti-inflammatory potential with values ranging from 73 to 90.35 %. All the other esters showed anti-inflammatory activity values ranging from 10.72 to 63.66% at 4th hour. Compound **7** was found to have the highest activity in the late phase of inflammation, i.e. 90.35%. Experimental model animals (albino rats) were observed alive without any abnormality after one month of experiment. The results are calculated and summarized in table 3.

## DISCUSSION

All the synthesized compounds were investigated for their antitumor activity by using potato disc antitumor assay. Potato disc antitumor assay is a prescreen assay and its results are in accordance with other most commonly used *in vitro* antitumor assays as mechanism of tumor induction are similar in both plants and animals ( Nawaz *et al*., 2009; Ahmad *et al*., 2008; Ullah *et al*., 2007). All the synthesized Schiff base esters (compounds **1-14**) were investigated against *Agrobacterium tumefaciens* (At 10) induced tumors in potato discs for their antitumor potential. Assay was carried out at different concentrations (10, 100 and 1000 µg/ml) of synthesized compounds to check whether concentration of compound influenced the tumor formation ability of bacterium and to calculate $IC_{50}$ values. As shown in table 2, 100% tumor was observed in case of vincristine (positive control) at all the concentrations tested whereas THF (negative control) did not interfere with the bacterium's activity to induce tumors. Furthermore, results indicate that the tumor inhibition was concentration dependant as highest tumor inhibition was observed at 1000 µg/ml concentration. Antitumor activity can also be determined by $IC_{50}$ value (50% inhibitory concentration which is calculated by using inhibition curves of all the concentrations tested for each test compound). Lower the value of $IC_{50,}$ more effective and active is the tested compound. In the present study, compound **9** exhibited best activity (69.30, 80.00 and 90.72% tumor inhibition at 10, 100 and 1000 µg/ml respectively) followed by compound **7** (55.48, 60.56, 88.97% tumor inhibition at 10, 100 and 1000 µg/ml respectively). These compounds (**9** and **7**) were further tested at lower concentrations to find their $IC_{50}$ values and these were found as most active antitumor agents with lowest $IC_{50}$ values i.e. 0.15 and 8.03 µg/ml respectively. Other compounds also showed significant tumor inhibitory activity (table 2). It had been observed that each concentration had significant ($P<0.05$) impact on tumor formation.

Anti-oxidant and pro-oxidant effects of the synthesized Schiff base esters were investigated *in vitro* qualitatively by using free radical induced oxidative DNA damage assay (Zaheer *et al*., 2010; Tian and Hua, 2005). In Fenton reaction, $Fe^{2+}$ ions react with $H_2O_2$ and OH free radicals are formed. OH radicals are considered to cause the damage to biomolecules (Vrchovska *et al*., 2006; Behl and Moosmann, 2002). DNA damage/protecting activity of all newly synthesized compounds (Schiff base esters) was investigated by *in vitro* free radical induced DNA damage system at various concentrations (10, 100 and 1000 µg/ml). Plasmid pBR322 DNA naturally exists in super-coiled form (SC). As a result of attack of OH

**Table 3**: Percentage edema inhibition by the Schiff base esters in carrageenan induced edema test in rat hind paw

| Compd. | % edema inhibition [A, B, C] | | | |
|---|---|---|---|---|
| | First Hour | Second Hour | Third Hour | Fourth Hour |
| **1** | 26.59 ± 2.90 | 30.93 ± 9.90 | 23.07 ± 3.75 | 37.39 ± 3.80 |
| **2** | 21.63 ± 11.71 | 11.97 ± 9.46 | 23.38 ± 6.85 | 10.72 ± 1.76 |
| **3** | 40.95 ± 5.64 | 35.31 ± 0.76 | 7.23 ± 4.89 | 18.26 ± 3.54 |
| **4** | 33.51 ± 4.85 | 42.81 ± 9.96 | 41.84 ± 9.0 | 18.26 ± 0.70 |
| **5** | 88.86 ± 2.1 | 55 ± 2.04 | 49.42 ± 3.6 | 42.91 ± .01 |
| **6** | 62.85 ± 11.72 | 66.28 ± 8.41 | 58.65 ± 1.06 | 80.38 ± 6.25 |
| **7** | 51.42 ± 7.66 | 64.36 ± 5.89 | 74.20± 12.32 | 90.35 ± 7.73 |
| **8** | 46.80 ± 4.34 | 22.65 ± 5.69 | 13.69 ± 3.39 | 23.91 ± 6.03 |
| **9** | 51.42 ± 12.86 | 52.49 ± 6.77 | 68.55 ± 8.48 | 78.13 ± 7.28 |
| **10** | 45.71 ± 18.15 | 48.27 ± 4.78 | 55.12 ± 4.51 | 72.99 ± 7.79 |
| **11** | 10.28 ± 17.15 | 4.06 ± 9.92 | 1.53 ± 9.38 | 16.52 ± 6.33 |
| **12** | 49.71 ± 16.86 | 40.99 ± 1.38 | 32.86± 4.59 | 76.20 ± 3.73 |
| **13** | 48.57 ± 4.84 | 54.40 ± 7.86 | 63.60 ± 4.54 | 74.91 ± 7.73 |
| **14** | 31.42 ± 10.19 | 33.33 ± 13.71 | 42.75± 18.12 | 63.66 ± 14.53 |
| **[*]+ve control** | 78.28±7.9 | 59.77± 9.5 | 74.91 ± 6.1 | 85.53 ± 3.6 |

[A]Data represents the mean of triplicate, [*]+ve control = Diclofenac potassium
[B]± Standard error to mean, [C]% edema inhibition = (1-VS / VN)*100
Where VN = Mean increase in volume of paw in negative control and VS = Mean increase in volume of paw in test samples.

radicals formed in Fenton reaction, if scission occurs on one strand (single stranded nicking) of plasmid DNA, the supercoiled (SC) form will be relaxed to generate a slow moving open circular (OC) form. If both strands of plasmid pBR322 DNA are cleaved (double stranded nicking), a linear form that migrates between open circular (OC) form and supercoiled (SC) form will be generated. Thus the ability of test compounds to unwind or condense a supercoiling substrate such as plasmid DNA was examined. Anti-oxidant or pro-oxidant effects of compounds on pBR322 DNA were evaluated on the basis of increase or loss of percentage of supercoiled band, when compared with the negative control value. In the present study, it was found that all the synthesized compounds showed plasmid DNA protection (antioxidant effect) at different concentrations (fig. 1). In negative control (X), maximum damage to DNA can be seen due to exposure of plasmid DNA to $FeSO_4$ along with $H_2O_2$. Lane 1-9 corresponds to plasmid DNA treated with the test compounds (compound **5**, **9** and **13**); here the bands of super coiled plasmid DNA were thicker than the bands of plasmid DNA in open circular form, which indicated an antioxidant effect of compounds on DNA. Moreover it was noted that the compounds showed antioxidant effect on DNA in dose dependent manner i.e. antioxidant effect on DNA increased with increase in concentration of the compound.

In the present study, carrageenan induced edema test in rat hind paw was used to determine anti-inflammatory effect of the newly synthesized Schiff base esters *in vivo* (Yaqub *et al*., 2009). In the early phase of inflammation, i.e. at first hour, compound **5**, **6**, **7** and **9** showed more than 50 % anti-inflammatory potential ranging from 51.42 to 88.86 % (Table 3). Early phase of inflammation is induced by histamine and various kinins (Yaqub *et al*., 2009). Anti-inflammatory activity shown by Schiff base esters during early phases of inflammation might be due to inhibition of these mediators. In the late phase of inflammation, compounds **6**, **7**, **9**, **10**, **12** and **13** showed more than 70 % anti-inflammatory potential with values ranging from 73 to 90.35%. All the other esters showed anti-inflammatory activity values ranging from 10.72 to 63.66% at 4$^{th}$ hour. Compound **7** was found to have the highest activity in the late phase of inflammation, i.e. 90.35% (table 3). The second or late phase of inflammation is mediated by prostaglandins and other slow reacting chemicals which peak at 3$^{rd}$ hour. Anti-inflammatory activity exhibited by test compounds during late phases of inflammation might be due to the inhibitory effect of test compounds on prostaglandins. Experimental model animals (albino rats) were observed alive without any abnormality after one month of experiment.

***Quantitative structure activity relationship (QSAR)***

Compounds no **1**, **2**, **3**, **4**, **6**, **7** and **8** have same R group i.e. H but these are different in R' group i.e. 4-lauroyloxyaniline, 4-myristoyloxyaniline, 4-(4″-methyl) benzoyloxyaniline, 4-[2″-{4‴-(2⁗-methyl propyl) phenyl}] propanoyloxyaniline, 4-(3″-phenyl) butyroyl-oxyaniline, 4-naphthoyloxyaniline and 4-cinnamoylo-xyaniline respectively. Presence of 4-(4″-methyl) benzoyloxyaniline) (compound **3**) and 4-cinnamoylo-xyaniline (compound **8**) resulted in decrease in tumor inhibition activity ($IC_{50}$ values 297.02 and 170.47 µg/ml respectively), while all other Schiff base esters which have same R group (H) showed significant antitumor activities with $IC_{50}$ values ranging from 8.03-36.20 µg/ml. Compound **6** (4-(3″-phenyl)butyroyloxyaniline) and **7** (4-naphthoyloxyaniline) also exhibited good anti-inflamma-tory activities in carrageenan induced edema test in rat hind paw i.e**.** 80.38 and 90.35% inhibition (highest among all compounds tested) respectively in late phase of inflammation. Compound **3** (4-(4″-methyl)benzoylo-xyaniline) and **4 (**4-[2″-{4‴-(2⁗-methyl propyl) phenyl}] propanoyloxyaniline) although differently substituted, did not show any significant difference in their activity in this particular assay (18.26% inhibition). Compound **8** showed non significant activity in anti-inflammatory assay as well (23.91% inhibition).

Increasing the number of carbon atoms in the alkyl side chain of compound **2** (14 C) resulted in decrease in antitumor activity ($IC_{50}$ value 36.20 µg/ml) when compared with compound **1** with 12 C which has $IC_{50}$ value 14.47 µg/ml. Similar behavior of these compounds were observed in anti-inflammatory assay where compound **1** (12 C) exhibited more edema inhibition (37.39%) than compound **2** (14 C) with 10.72% inhibition in late phase of inflammation.

Compound **5**, **9** and **10** have same R group i.e. $OCH_3$ but different R' groups i.e. 2″-{4‴-(2⁗-methylpropyl)phenyl} propanoyloxyaniline, 4-(3″-phenyl)butyroyloxyaniline and 4-naphthoyloxyaniline respectively. These compounds exhibited best antitumor activity among all the tested compounds and had the $IC_{50}$ values ranging from 0.157 - 15.40 µg/ml respectively. It was observed that compound **9** exhibited best tumor inhibition activity with lowest $IC_{50}$ value i.e. 0.157 µg/ml (lowest among all of the synthesized compounds) due to presence of 4-(3″-phenyl)butyroyloxyaniline R' group, presence of 2″-{4‴-(2⁗-methylpropyl)phenyl} propanoyloxyaniline (compound **5**) decreased the tumor inhibition activity ($IC_{50}$ value 13.57 µg/ml) and presence of 4-naphthoyloxyaniline (compound **10**), further decreased the tumor inhibition activity ($IC_{50}$ value 15.40 µg/ml). Compound **5** with 2″-{4‴-(2⁗-methylpropyl)phenyl} propanoyloxyaniline also exhibited excellent activity during early phase of inflammation 88.86% inhibition in anti-inflammatory assay, while compound **9** and **10** have more then 70% inhibition in late phase of inflammation i.e. 78.13 and 72.99% respectively.

Compound **11** and **12** have common R group i.e. Cl but different R' group i.e. 4-lauroyloxyaniline and 4-naphthoyloxyaniline respectively. Compound **11** showed comparatively better tumor inhibition activity ($IC_{50}$ value 34.75 µg/ml) than compound **11** (**$IC_{50}$** value 113.92 µg/ml) but in case of anti-inflammatory assay results were *vice versa* i.e. 16.52% inhibition by compound **11** and 76.20% inhibition by compound **12** in late phase of inflammation.

Compound **13** and **14** have common R group i.e. $CH_3$ but different R' group i.e. 4-(3″-methyl) benzoyloxyaniline and 4-(3″-phenyl)butyroyloxyaniline respectively. Compound **14** resulted in better tumor inhibition activity ($IC_{50}$ value 18.98 µg/ml) than compound **13** ($IC_{50}$ value 204.08 µg/ml). In anti-inflammatory assay, compound **13** and **14** although differently substituted, did not show significant variation in their activity (74.91 and 63.66% respectively).

## CONCLUSION

Some Schiff bases were converted to esters through two practical routes in good yields. To evaluate the pharmacological potential of synthesized compounds, all the synthesized Schiff base esters were subjected to potato disc antitumor assay, free radical induced oxidative DNA damage analysis and carrageenan induced edema test in rat hind paw. In conclusion, all the synthesized compounds showed significant tumor inhibitory activities. Compound **9** and **7** showed best tumor inhibition activity ($IC_{50}$ value 0.15 and 8.03 µg/ml respectively) in potato disc antitumor assay. All the synthesized compounds (Schiff base esters) exhibited antioxidant effect on plasmid DNA at all the concentrations tested in free radical induced DNA damage analysis. Compound **5** was found to be most active compound in the early phase of inflammation (88.86% inhibition) and compound **7** was found to have the highest activity in the late phase of inflammation, i.e. 90.35%. We expect these compounds as potential candidates for preparation of a wide variety of pharmaceutically active agents.

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