Chapter-3

Nanostructured TiO₂ catalyzed microwave assisted synthesis of fused Quinolines/Benzo[h]quinolines: DNA Binding, Molecular docking and Antioxidant activity

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3.1. Introduction

In recent years, the metal oxide semiconductor photocatalysis using particulate TiO\textsubscript{2} has proven to be a promising technology for use in photocatalytic reactions, in the cleanup of water contaminated with hazardous industrial by-products and even in many organic conversions [1-6], or as a photoactive material in nanocrystalline solar cells [7-13]. To produce chemical energy and catalytic activity, it is important to prevent the electron from recombining with the valence band hole [1]. On the other hand, the significant examples of organic transformations employed for synthetic purposes are oxidation and reduction reactions, isomerization reactions, C–H bond activations, C–C and C–N bond forming reactions [2-6]. Titanium dioxide, could be the photocatalyst of considerable interest because of its photocatalytic properties and choice for a large variety of applications because it is cheap, non-toxic, and has redox properties which are favorable both for oxidation of many organics and for reduction of a number of metal ions or organics in any organic/aqueous media.

Quinolines and their derivatives are very important class of compounds because of their wide occurrence in natural products [7] and biologically active compounds [8]. Furthermore, poly-substituted quinolines have been found to undergo hierarchical self assembly into a variety of nano- and mesostructures with enhanced electronic and photonic functions [9]. In view of these points, a great deal of effort has been drawn to develop new and efficient synthetic routes to quinoline derivatives in both synthetic organic and medicinal chemistry.

The use of scientific microwave apparatus in the development of efficient and selective greener methods has become a major focus of research worldwide and selection of appropriate alternate eco-friendly reaction media has become an integral part of this
paradigm shift [10]. Microwave (MW) irradiation as an alternative energy source in conjunction with water as reaction medium has proven to be a successful 'greener' chemical approach.

The literature survey reveals that there is evidence to infer the antitumor activity is due to the intercalation between the base pairs of DNA and interferences with the normal functioning of enzyme topoisomerase II, which is involved in the breaking and releasing of DNA strands [11]. The antitumor drugs that intercalate DNA are of growing interest in the field of anticancer drugs. Particularly, they are characterized by planar chromospheres, which are often constituted by three or four condensed rings, which can intercalate into base pairs. Results of the various binding studies have been useful in designing new and promising anticancer agents [12]. The DNA binding studies of pyrimidothienoquinolines have been recently reported [13,14].

In order to demonstrate the potential antioxidant activity of heterocyclic compounds, the interactions of nitrogen, sulfur and oxygen containing molecules with DNA are of major biochemical and biological importance [15]. It has been suggested that quinolines can chelate Fe(II) or Fe(III) and prevent free radical production in Fenton reaction and quinolines themselves can also intercalate DNA duplex and react with free radicals in order to protect DNA from oxidative damage [16,17].

Our approach is to develop a rapid and efficient scientifically based framework for greener preparation of carbonitrile quinoline/benzoquinolines in a manner that renders the materials less mobile in the environment and reduces or eliminates the use and generation of hazardous substances. The areas of opportunity are being exploited to engage green chemistry: (i) choice of solvent, (ii) the catalytic agent employed, hence, in the present chapter the author describes the microwave (MW) irradiation as an efficient
and selective mode of activation in rapid and efficient synthesis of 2-hydroxy-4-phenyl/benzo[h]quinoline-3-carbonitrile and 2H-pyrano/2H-thiopyrano[2,3-b]quinoline-2-carboxylic acids by using nanostructured TiO2 catalyst. And also, the in vitro antioxidant activity, docking and interaction study with ds-DNA is presented.

3.2. Materials and Method

3.2.1. Chemicals and instruments

All chemicals were AR grade and purchased commercially. The instruments used for characterization were mentioned in (Chapter 2; Section 2.2.1). The preparation part of TiO2 is presented in (Chapter 2; Section 2.2.2). Tris-HCl buffer, ferric chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trichloracetic acid (TCA) were purchased from Sigma (Sigma-Aldrich, E. Merck, Himedia, Qualigens, Mumbai, India), Calf thymus DNA (CT-DNA) and pUC19 DNA were purchased from Bangalore Gene, Bangalore, India and solution was prepared using deionised double distilled water.

3.2.2. General experimental procedure for the synthesis of quinoline/benzo[h]quinoline-3-carbonitrile (4a-c and d)

A dry, 50-mL flask was charged with arylaldehyde (5 mmol), cyano acetate (5 mmol), aniline (5 mmol), and titanium dioxide (TiO2) nanopowder (500 mg). The mixture was well mixed and then irradiated in a SANYOEM-350S microwave oven at 150W for a designated time (1-2 min) as required for completing the reaction (determined by TLC). Then, after being cooled to room temperature, under these
conditions, the reaction mass was poured on crushed ice, the solid compounds were filtered off and the crude product was purified by recrystallization from DMF. The similar reaction procedure has been followed for other derivatives.

**Physico-chemical spectral details of 2-hydroxy-4-phenylquinoline-3-carbonitrile (4a)**

Pale red solid. Yield 85%, mp.132 °C; IR (v) (KBr) cm⁻¹; 2019 (C-H, Ar-H); 2224 (C=N); 3278 (OH); ¹H NMR (DMSO de), δ 7.65 (d, 1H, Ar-H, J = 8.03), 7.58 (d, 1H, Ar-H, J = 7.63), 7.36 (d, 1H, Ar-H, J = 7.45), 7.94 (d, 1H, Ar-H, J = 8.77), 7.24 (d, 1H, Ar-H, J = 7.13), 7.27 (d, 1H, Ar-H, J = 7.35), 7.47 (d, 1H, Ar-H, J = 7.37), 7.45 (d, 1H, Ar-H, J = 7.37), 7.34 (d, 1H, Ar-H, J = 7.47), 11.2 (s, 1H, O-H, J = 11.03); m/z (%) [M]+: [246]+; Elemental analysis, Found: C, 78.04; H, 4.08; N, 11.37. Calculated for C₁₀H₁₀N₂O: C, 78.03; H, 4.09; N, 11.38.

**2-hydroxy-6-methyl-4-phenylquinoline-3-carbonitrile (4b)**

Brown solid, Yield 68%, mp.121 °C; IR (v) (KBr) cm⁻¹; 2025 (C-H, Ar-H); 2221 (C=N); 3298 (OH); 2950 (C-H of CH₃); ¹H NMR (DMSO de), δ 7.43 (d, 1H, Ar-H, J = 7.68), 7.45 (d, 1H, Ar-H, J = 7.61), 7.85 (d, 1H, Ar-H, J = 8.05), 7.48 (d, 1H, Ar-H, J = 7.26), 7.32 (d, 1H, Ar-H, J = 7.26), 7.22 (d, 1H, Ar-H, J = 7.26), 7.32 (d, 1H, Ar-H, J = 7.26), 7.48 (d, 1H, Ar-H, J = 7.26), 7.34 (d, 1H, Ar-H, J = 7.47), 2.36 (d, 3H, CH₃), 11.5 (s, 1H, O-H, J = 11.03); m/z (%) [M]+: [260]+; Elemental analysis, Found: C, 78.43; H, 4.66; N, 10.77. Calculated for C₁₇H₁₂N₂O: C, 78.44; H, 4.65; N, 10.76.

**2-hydroxy-6-methoxy-4-phenylquinoline-3-carbonitrile (4c)**

Brown solid, Yield 76%, mp.112 °C; IR (v) (KBr) cm⁻¹; 2021 (C-H, Ar-H); 2236 (C=N); 3284 (OH); 1250 (C-O-C of OCH₃); ¹H NMR (DMSO de), δ 6.94 (d, 1H, Ar-H, J
= 7.68), 7.31 (d, 1H, Ar-H, J = 7.61), 7.84 (d, 1H, Ar-H, J = 8.05), 7.50 (d, 1H, Ar-H, J = 7.26), 7.35 (d, 1H, Ar-H, J = 7.26), 7.28 (d, 1H, Ar-H, J = 7.26), 7.28 (d, 1H, Ar-H, J = 7.26), 7.54 (d, 1H, Ar-H, J = 7.26), 3.73 (d, 3H, OCH3), 11.2 (s, 1H, O-H, J = 11.03); m/z (% [M]+ : [276]+; Elemental analysis. Found: C, 73.91; H, 4.39; N, 10.13. Calculated for C17H12N2O: C, 73.90; H, 4.38; N, 10.14.

2-hydroxy-4-phenylbenzo[h]quinoline-3-carbonitrile (d)

Dark violet solid. Yield 81%, mp.151 °C ; IR (v) (KBr) cm⁻¹: 3015 (C-H, Ar-H); 2225 (C=N); 3271 (OH); ¹H NMR (DMSO d₆), δ 7.64 (d, 1H, Ar-H, J = 7.68), 7.35 (d, 1H, Ar-H, J = 7.43), 7.67 (d, 1H, Ar-H, J = 7.67), 7.32 (d, 1H, Ar-H, J = 7.32), 7.34 (d, 1H, Ar-H, J = 7.32), 7.72 (d, 1H, Ar-H, J = 7.68), 7.48 (d, 1H, Ar-H, J = 7.26), 7.32 (d, 1H, Ar-H, J = 7.26), 7.22 (d, 1H, Ar-H, J = 7.26), 7.32 (d, 1H, Ar-H, J = 7.26), 7.48 (d, 1H, Ar-H, J = 7.26), 11.8 (s, 1H, O-H, J = 11.03); m/z (% [M]+ : [296]+; Elemental analysis. Found: C, 81.08; H, 4.07; N, 9.46. Calculated for C20H12N2O: C, 81.07; H, 4.08; N, 9.45.

3.2.3. General procedure for the synthesis of 5-methyl-3,4-dihydro-2H-pyrano[2,3-b]quinoline-2-carboxylic acid (8a-8a/9b-9b2)

The mixture of 2-hydroxy/2-mercapto-4-methylquinoline (0.01M) and maleic anhydride (0.01M) in the presence of TiO₂ (0.02M) and ammonium acetate (0.02M) in absolute ethanol was irradiated in microwave oven at 110 °C for 1-2 mins. The reaction was monitored by TLC, the reaction mixture was poured on crushed ice. The obtained solid was filtered and recrystallized with methanol.
5-Methyl-3,4-dihydro-2H-pyran[2,3-b]quinoline-2-carboxylic acid (8a)

Brown solid, Yield 85%, m.p. 192-194 °C; IR (v) (KBr) cm⁻¹; 3015 (C=H, Ar-H); 1658 (C=N); 1278 (COOH); ¹H NMR (DMSO d₆), δ 7.95 (d, 1H, Ar-H, J = 8.03), 7.75 (d, 1H, Ar-H, J = 7.63), 7.56 (d, 1H, Ar-H, J = 7.45), 7.26 (d, 1H, Ar-H, J = 7.37), 4.61 (d, 1H, C-H, J = 2.12), 2.26 (t, 2H, CH₂, J = 1.41), 2.31 (t, 2H, CH₂, J = 1.41), 2.42 (d, 3H, CH₃), 11.2 (s, 1H, O-H, J = 11.03); [M]+ : [243]+; Elemental analysis, Found: C, 69.13%; H, 5.37%; N, 5.78%. Calculated for C₁₄H₁₃NO₃: C, 69.12%; H, 5.39%; N, 5.76%.

5,7-Dimethyl-3,4-dihydro-2H-pyran[2,3-b]quinoline-2-carboxylic acid (8a₁)

Brown solid, Yield 72%, m.p. 174-177 °C; IR (v) (KBr) cm⁻¹; 3021 (C=H, Ar-H); 1667 (C=N); 1271 (COOH); ¹H NMR (DMSO d₆), δ 7.83 (d, 1H, Ar-H, J = 8.03), 7.78 (d, 1H, Ar-H, J = 7.63), 7.51 (d, 1H, Ar-H, J = 7.45), 3.26 (s, 3H, CH₃), 4.67 (d, 1H, C-H, J = 2.12), 2.26 (t, 2H, CH₂, J = 1.41), 2.35 (t, 2H, CH₂, J = 1.41), 2.41 (d, 3H, CH₃), 11.2 (s, 1H, O-H, J = 11.03); [M]+ : [257]+; Elemental analysis, Found: C, 71.02%; H, 5.87%; N, 5.46%. Calculated for C₁₅H₁₅NO₃: C, 70.02%; H, 5.88%; N, 5.44%.

7-Methoxy-5-methyl-3,4-dihydro-2H-pyran[2,3-b]quinoline-2-carboxylic acid (8a₂)

Brown solid, Yield 75%, m.p. 204-206 °C; IR (v) (KBr) cm⁻¹; 3015 (C=H, Ar-H); 1668 (C=N); 1283 (COOH); ¹H NMR (DMSO d₆), δ 7.81 (d, 1H, Ar-H, J = 8.03), 7.75 (d, 1H, Ar-H, J = 7.63), 7.56 (d, 1H, Ar-H, J = 7.45), 3.42 (s, 3H, CH₃), 4.66 (d, 1H, C-H, J = 2.12), 2.29 (t, 2H, CH₂, J = 1.41), 2.36 (t, 2H, CH₂, J = 1.41), 2.51 (d, 3H, CH₃), 11.2 (s, 1H, O-H, J = 11.03); [M]+ : [273]+; Elemental analysis, Found: C, 65.91%; H, 5.54%; N, 5.14%. Calculated for C₁₅H₁₃NO₄: C, 65.92%; H, 5.53%; N, 5.13%.
5-Methyl-3,4-dihydro-2H-thiopyrano[2,3-b]quinoline-2-carboxylic acid (9b)

Dark yellow solid, Yield 85%, m.p. 192-195 °C; IR (ν) (KBr) cm⁻¹; 3026 (C-H, Ar-H); 1652 (C=N); 1286 (COOH); ¹H NMR (DMSO d₆), δ 7.96 (d, 1H, Ar-H, J = 8.05), 7.78 (d, 1H, Ar-H, J = 7.61), 7.52 (d, 1H, Ar-H, J = 7.46), 7.23 (d, 1H, Ar-H, J = 7.45), 4.64 (d, 1H, C-H, J = 2.14), 2.24 (t, 2H, CH₂, J = 1.40), 2.34 (t, 2H, CH₂, J = 1.42), 2.47 (d, 3H, CH₃), 11.5 (s, 1H, O-H, J = 11.01); [M⁺]:[259]⁺; Elemental analysis, Found: C, 64.85 %; H, 5.03 %; N, 5.42 %. Calculated for C₁₄H₁₃NO₂S: C, 64.84 %; H, 5.05 %; N, 5.40 %.

5,7-Dimethyl-3,4-dihydro-2H-thiopyrano[2,3-b]quinoline-2-carboxylic acid (9b₁)

Brown solid, Yield 78 %, m.p. 185-187 °C; IR (ν) (KBr) cm⁻¹; 3029 (C-H, Ar-H); 1642 (C=N); 1281 (COOH); ¹H NMR (DMSO d₆), δ 7.98 (d, 1H, Ar-H, J = 8.05), 7.72 (d, 1H, Ar-H, J = 7.61), 7.59 (d, 1H, Ar-H, J = 7.46), 2.35 (s, 3H, CH₃), 4.53 (d, 1H, C-H, J = 2.14), 2.25 (t, 2H, CH₂, J = 1.40), 2.31 (t, 2H, CH₂, J = 1.42), 2.44 (d, 3H, CH₃), 11.9 (s, 1H, O-H, J = 11.01); [M⁺]:[273]⁺; Elemental analysis, Found: C, 65.92 %; H, 5.52 %; N, 5.13 %. Calculated for C₁₅H₁₅NO₂S: C, 65.91 %; H, 5.53 %; N, 5.12 %.

7-Methoxy-5-methyl-3,4-dihydro-2H-thiopyrano[2,3-b]quinoline-2-carboxylic acid (9b₂)

Dark yellow solid, Yield 80 %, m.p. 178-180 °C; IR (ν) (KBr) cm⁻¹; 3021 (C-H, Ar-H); 1645 (C=N); 1285 (COOH); ¹H NMR (DMSO d₆), δ 7.91 (d, 1H, Ar-H, J = 8.05), 7.75 (d, 1H, Ar-H, J = 7.61), 7.48 (d, 1H, Ar-H, J = 7.46), 3.63 (s, 3H, OCH₃), 4.56 (d, 1H, C-H, J = 2.14), 2.29 (t, 2H, CH₂, J = 1.40), 2.36 (t, 2H, CH₂, J = 1.42), 2.48 (d, 3H, CH₃), 11.6 (s, 1H, O-H, J = 11.01); [M⁺]:[289]⁺; Elemental analysis, Found: C, 62.25 %; H, 5.24 %; N, 4.85 %. Calculated for C₁₅H₁₅NO₃S: C, 62.26 %; H, 5.23 %; N, 4.84 %.
3.2.4. Docking studies

The ligand structures (3D) were constructed, pre-optimized with implemented MM2 force field and further refined using Vega 1.5 [18]. The PM3 method was used for geometry optimization and charge calculation. To allow flexibility in the ligand, it is necessary to assign the routable bonds; however in this case all the rotamers were allowed.

The PDB file of the selected structure 'DSC was downloaded from the database (www.rcsb.org/pdb) and the ligand was removed from the fragment. Docking of the ligands into receptor was carried out using AutoDock (version 3.0.5) set of programs [19]. The resulting macromolecule was setup for docking as follows: polar hydrogens were added using the PROTONATE utility. Solvation parameters were added to the final protein file using the ADDSOL utility of AutoDock. It was carried out using the empirical free energy function and the Lamarckian genetic algorithm (LGA) [20] applying a standard protocol, with an initial population of 100 randomly placed individuals, a maximum number of $1.5 \times 10^6$ energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. Proportional selection was used, where the average of the worst energy was calculated over a window of the previous 10 generations. For the local search, the so-called pseudo-Solis and Wets algorithm [21] was applied using a maximum of 300 iterations per local search. Ten independent docking runs were carried out for each ligand. At the end of each AutoDock execution, in which more than one run was performed, the program outputs a list of clusters and their energies. The clustering of docked conformations is determined by the tolerance specified in Å.
3.2.5. DNA binding studies (Absorption Spectroscopy)

The UV-Vis spectra were recorded on SHIMADZU, UV-1650 PC model spectrophotometer. The absorbance assessments were performed at pH 7.3 by keeping the concentration of DNA constant (0.25 mM), while varying the concentration of compounds 8a/9b. The values of the binding constants \( K_b \) were obtained according to the methods reported [22,23].

3.2.6. Viscosity measurements

Viscosity measurements were carried out by using a semimicro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S at 20 °C. For the viscosity experiments, samples of calf thymus DNA were sonicated [24] to fragments having an estimated molecular weight of approximately [25,26].

3.2.7. Thermal denaturation experiments

The DNA melting studies were done by controlling the temperature of the sample cell with a Shimadzu (SHIMADZU, UV-1650 PC) circulating bath while monitoring the absorbance at 260 nm. The temperature of the solution was continuously monitored with a thermo-couple attached to the sample holder.

3.2.8. In vitro antioxidant activity

3.2.8.1. Superoxide anion scavenging activity

Measurements of superoxide anion scavenging activity were based on the method described [27]. Superoxide radicals were generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM,
pH 8.0) containing 1mL of NBT (50 mM) solution, 1mL NADH (78 mM) solution, and a sample solution of complexes (20, 40, 60 μg/mL) in ethanol. The reaction was started by adding 1mL of phenazine methosulfate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance was recorded at 560 nm. L-Ascorbic acid was used as a control. The percentage inhibition of superoxide anion generation was calculated using the formula shown in Eqn. (1) [28].

\[
\% \text{ Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]  

Where \( A_0 \) was the absorbance of the control reaction and \( A_1 \) was the absorbance in the presence of the samples 8a and 9b.

3.2.8.2. Scavenging of hydrogen peroxide

The ability of the scavenging effect of 8a and 9b to hydrogen peroxide was determined according to the reported method [29]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Complexes (20, 40, 60 μg/mL) in ethanol and distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution. The percentage of scavenging of hydrogen peroxide was calculated using the equation (1):

\[
\% \text{ Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

3.2.8.3. Hydroxyl radical (HO·) scavenging assay

The ability of compounds to scavenge the hydroxyl radical generated by the Fenton reaction was measured according by modified method [30]. A 200 μL of 10 mM FeSO₄·7H₂O, 200 μL of 10 mM EDTA and 200 μL of 10 mM 2-deoxyribose were mixed
with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 μL of different concentration of compounds. Thereafter, 200 μL of 10 mM H₂O₂ was added to the mixture before incubation for 4 h at 37 °C. Later, 1mL of 2.8% TCA and 1mL of 1% TBA were added and placed on a boiling water bath for 10 min. Absorbance was recorded at 532 nm.

3.2.8.4. DPPH free radical scavenging activity

The free radical scavenging activity was measured against 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method [31]. Briefly, 1 ml of 0.1mM solution of DPPH in ethanol was added to 3mL of compounds in phosphate buffer at different concentrations (20, 40, 60 μg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min and then the absorbance was measured at 517 nm. The DPPH concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R²: 0.9769):

\[
\text{Absorbance} = 104.09 \times [\text{DPPH}^*]
\]

The DPPH radical concentration was calculated using the following equation (2):

\[
\text{DPPH Scavenging Effect} (%) = 100 - \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad (2)
\]

Where \( A_0 \) was the absorbance of the control reaction and \( A_1 \) was the absorbance in the presence of the sample, 8a or 9b.

3.2.8.5. Reducing power

The reducing power of newly synthesized compounds was determined according to the literature method [32]. The five concentrations of compounds (20-60 μg/mL) in 1mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and
potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%). The absorbance was measured at 700 nm.

3.3. Results and Discussion

3.3.1. Chemistry of TiO₂ nanoparticles

In this particular work, mixed-phase titanium dioxide (TiO₂) has been the primary photocatalyst of interest. The studies showed that the mixtures of anatase and rutile TiO₂ perform better than either individual rutile or anatase phases of TiO₂ [33,34].

Our approach was to develop

(i) The performance of mixed-phase TiO₂ nanoparticles as catalyst in synthesis of quinoline derivatives by microwave irradiation method (1-2 mins).

\[
\begin{align*}
\text{TiO}_2 & \xrightarrow{\text{Redn}} \text{TiO}_2\gamma + \frac{\gamma}{2}\text{O}_2 \\
\text{Oxidation} & \end{align*}
\]

(ii) A rapid, efficient and scientifically based framework for greener preparation of these quinolines in a manner that renders the materials less mobile in the environment and reduces or eliminates the use and generation of hazardous substances.

The areas of opportunity exploited to engage green chemistry are: (a) choice of solvent, (b) the catalytic agent employed; and finally, we found that microwave (MW) irradiation as an efficient and selective mode of activation for rapid and efficient synthesis of quinoline carboxylic acids.
The required colloidal TiO$_2$ was prepared according to reported procedure by a dropwise addition of titanium(IV) tetrachloride to water and cooled to 4 °C. The temperature and component mixing of reactants were controlled by an apparatus developed for automatic colloid preparation [35]. The photo-catalytic activity of TiO$_2$ was applied to synthesize biological molecules via microwave irradiation. The main drawback for using TiO$_2$ itself for catalytic action is that it absorbs light energy in the UV part of the spectrum. It has been found that surface atoms of TiO$_2$ are under coordinated [36] with organic molecules and provide an opportunity for alternating the effective band gap of nanoparticles. In the typical multicomponent reaction, the aldehyde, ethyl cyanoacetate and anilines results, substituted quinolines within 30-60 seconds in presence of TiO$_2$ catalyst. From the experimental results, it can be seen that the generality of this facile condensation was established in the presence of titanium dioxide nano-surface under solvent-free conditions to furnish the corresponding quinolines. By comparison, substrate 4a-c and d demonstrates a superior reaction activity to its conventional method of approach which consumed nearly 39-50 mins for completion of reaction (Scheme 3.1 and 3.2), in which the employed microwave output is only 150 W. Because the foregoing optimization work has demonstrated that this kind of reaction can also be efficiently promoted by conventional heating under neat conditions, all the above products obtained under solvent-free conditions. The general reaction mechanism for both reactions was shown in Scheme 3.3. The spectral data is described in experimental section-3.2.2 and the spectra are presented in figures 3.1-3.4.
Table 3.1: Reaction time data of conventional and MW methods.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conventional (min)</th>
<th>MW (min)</th>
</tr>
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<tbody>
<tr>
<td>4a</td>
<td>45</td>
<td>1-2</td>
</tr>
<tr>
<td>4b</td>
<td>48</td>
<td>2.0</td>
</tr>
<tr>
<td>4c</td>
<td>39</td>
<td>1-2</td>
</tr>
<tr>
<td>d</td>
<td>50</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Scheme 3.1: Synthetic route for quinoline-3-carbonitriles (R₁ = H, CH₃, and OCH₃)

Scheme 3.2: Synthetic route for benzo[h]quinoline-3-carbonitrile

Scheme 3.3: Describes the probable mechanism for both 2-hydroxy-4-phenyl/benzo[h]quinoline-3-carbonitrile
Figure 3.1: IR spectra of 2-hydroxy-4-phenylquinoline-3-carbonitrile (4a)
Figure 3.2: $^1H$ NMR spectra of benzo[h]quinoline-3-carbonitrile (d)
Figure 3.3: Mass spectra of 2-hydroxy-4-phenylbenzo[h]quinoline-3-carbonitrile (d)
**Figure 3.4:** Mass spectra of 2-hydroxy-4-phenylquinoline-3-carbonitrile (4a)
Further, the TiO$_2$ nanoparticles are used in the synthesis of 2H-pyran/2H-thiopyrano[2,3-b]quinoline-2-carboxylic acids (8a/9b). The substituted 2-hydroxy/mercaptoquinolines treated with maleic anhydride in presence of TiO$_2$ leads the desired products. A major advantage of TiO$_2$ catalyzed method was the involvement of fastest kinetics during the reaction. The product was confirmed by the IR, $^1$H NMR and mass spectral studies as presented in experimental section-3.2.3 (Scheme 3.4). The observed absorption frequencies at 1278 and 1286 cm$^{-1}$ (COOH) in IR spectra, and singlet at δ 11.00 and δ 10.80 in $^1$H NMR spectra are due to presence of carboxylic (COOH) functional groups. Finally, the structure was confirmed by its mass spectrum through the appearance of molecular ion peak at m/z 289[M$^+$] and 336 [M$^+$] and the spectra are presented in figures 3.5-3.8.

![Scheme 3.4: Synthetic route carried out for the synthesis of quinolines 8a-8a$_2$ and 9b-9b$_2$.](image)
Figure 3.5: IR Spectra of 5-methyl-3,4-dihydro-2H-pyrano[2,3-b]quinoline-2-carboxylic acid (8a)
Figure 3.6: $^1$H NMR spectra of 5-methyl-3,4-dihydro-2H-pyrano[2,3-b]quinoline-2-carboxylic acid (8a)
Figure 3.7: Mass spectra of 5-methyl-3,4-dihydro-2H-pyrano[2,3-b]quinoline-2-carboxylic acid (8a)
Figure 3.8: Mass spectra of 7-Methoxy-5-methyl-3,4-dihydro-2H-thiopyrano[2,3-b]quinoline-2-carboxylic acid (9b₂)
3.3.2. Docking studies

Careful examination of B-DNA structures reveals the reason for the difference in the positioning in the two helical forms. The predicted binding of ligands to the DNA bases on the basis of the clustering of Cartesian coordinates is thus more directional than that expected from the ellipsoids derived from Fourier averaging. Calculation of the energy of a drug-DNA system entails the enumeration of a set of critical atoms on the drug that may interact with the ligand-binding sites around the DNA bases. Each of the potential hydrogen-bond donor or acceptor atoms on the drug is assigned a DNA-binding ellipsoid with complementary acceptor or donor properties. The partner ellipsoid is selected on the basis of the magnitude of interaction with the drug atom, i.e., the interaction score of lowest value. The number of interactions with DNA is limited by the hydrogen-bonding quotas of the unfulfilled proton donor and acceptor sites on the edges of the Watson-Crick base pairs.

The strong preference to minor-groove over major-groove binding (70%) obtained by the docking simulations is in agreement with available spectroscopic data [37]. The preference of minor-groove binding drugs for AT-rich sequences is ascribed to favorable hydrophobic and Vander Waal's interactions [38,39], which is in accordance with experimental data reported for quinoline and other DNA drugs [40-43]. Since, an atomic-resolution structure of synthesized compounds bound to DNA is not yet available, there is no much experimental evidence regarding the orientation and binding modes of parent compounds 8a and 9b when it was bound at the minor groove. According to two criteria, (a) prediction statistics and (b) energy ranking, the docking of quinolines to DNA results in both energetically favorable binding mode and binding site.
In all the cases, the parent compounds, 8a/9b was shown to assume a spatial arrangement in which the planar heterocyclic moiety intercalates in AT portion of the DNA sequence whereas the side chain lies close to the minor groove. **Figure 3.7 and 3.8** shows the DNA-binding mode for the most interesting compounds 8a/9b. In order to rationalize structure-binding capability relationships, for 8a/9b were calculated the inhibition constants, binding, and docking energies (**Table 1**) by considering the importance of drugs when two molecules with π electron systems form charge-transfer complexes, especially in the case of DNA-interactive compounds. Here, the compound 8a forms four hydrogen bonds via one nitrogen and three oxygen atoms with B chain each but in the case of 9b three hydrogen bonds have been formed; one through nitrogen and the other two through both oxygen atoms as shown in **Figure 3.9 and 3.10**.

**Figure 3.9**: View of various energy minimized docked structures of compound 8a and showing of AT rich interaction with DNA base pairs of d(CGCGAATTCCGCG) [NBT code: GLDB 05]
Figure 3.10: View of various energy minimized docked structures of compound 9b and showing of AT rich interaction with DNA base pairs of d(CGCGAATTCGCG) [NBT code: GLDB 05]

3.3.3. DNA-Binding studies

Primarily, the DNA binding was observed by following parameters: (i) electrostatic interactions with the negative charged nucleic sugar–phosphate structure which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA.
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Figure 3.11: (a) Absorption spectra for 8a/9b in Tris-HCl buffer upon addition of DNA. 8a/9b = 0.5 μM, [DNA] = 0-100 μM. Above absorption plots showing variation in absorption with increase [DNA].

(b) Inner graph of [DNA]/(ε_a-ε_t) v/s [DNA] for titration of DNA with 8a/9b.

Absorbance spectroscopy

Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with compounds [32,33]. If the binding mode is intercalation, the π* orbital of the intercalated compound can couple with the p orbital of the base pairs, thus, decreasing the π→π* transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism [43].

The compound's intercalation was evident from the major reduction in the intensity of UV-Vis bands characteristic of quinolines upon DNA interaction (Figure 3.11). The major intensity decrease at 375 nm (8a) and 370 nm (9b) is indicative of
DNA-drug intercalation (Figure 3.11). It has been demonstrated that intercalation of $8a$ and $9b$ into DNA duplex causes major reduction in the intensity of the UV–Vis absorption band characteristics of compounds. This hypochromic shift indicates helical ordering of $8a$ and $9b$ in the DNA helix. The limitation on molecular movements of both compounds causes a decrease in its ability to absorb light energy [45,46]. In addition, two isosbestic points at 302 for $8a$ and 325 nm for $9b$, indicated enhanced intercalation of the above compounds into DNA duplex. Absorption spectral results exhibit hypochromism at about 20.5 and 25.1%, for compounds $8a$ and $9b$, respectively. The binding constant value are given in Table 3.2, indicates that $3.5 \times 10^6$ for $8a$ $2.9 \times 10^5$ for $9b$.

Table 3.2: DNA binding constants ($K_b$) and DNA melting temperature ($T_m$) for $2a/2b$.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_b (M^{-1})$</th>
<th>$T_m (^\circ C)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$8a$</td>
<td>$3.5 \times 10^6$</td>
<td>60</td>
</tr>
<tr>
<td>$9b$</td>
<td>$2.9 \times 10^5$</td>
<td>58</td>
</tr>
</tbody>
</table>

3.3.4. Viscosity measurements

To further clarify, the interaction modes of the compounds $8a/9b$ with DNA were investigated by viscosity measurements. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process [47,48]. The measured viscosity changes in short, rod like DNA fragments. The relative length increase ($L/L_0$) of the complex formed between $8a/9b$ with DNA is shown in Figure 3.12. It is evident that binding of these compounds increased the viscosity of DNA corresponding to an increase in the contour length of the DNA fragments. The measured slopes of the plots $1.23 \pm 0.03$, for compound $8a$ falls within 63% and $1.05 \pm 0.025$ for $9a$ falls within 53% of the
slope of a theoretical curve for an idealized intercalation process \((1 + 2r)\) [49]. The results shows that intercalation of \(8a\) molecule provoked an increase of \(1.9\ \text{Å}\) in the contour length of DNA. Since, the size of these sonicated fragments was significantly greater than the persistence length; the estimated \(1.9\ \text{Å}\) lengthening is probably best regarded as a lower limit.

![Graph showing relative viscosity vs. [comp]/[DNA]](image)

**Figure 3.12:** Plot shows effect of \(8a/9b\) on the viscosity of CT-DNA at 25 \((±0.1^\circ\text{C})\).

\(8a/9b = 0-100\ \mu\text{M} \) and \([\text{DNA}] = 50\ \mu\text{M}\).

### 3.3.5. Thermal denaturation

Other strong evidence for the intercalative binding of compounds into the double helix DNA was obtained from DNA melting studies. The intercalation of small molecules into the double helix is known to increase the DNA melting temperature \((T_m)\), at which the double helix denatures into single stranded DNA owing to the increased stability of the helix in the presence of an intercalator [50]. The molar extinction coefficient of DNA bases at 260 nm in the double helical form is much lesser than the single stranded form; hence, melting of the helix leads to an increase in the absorbance at 260 nm. Thus, the helix to coil transition temperature can be determined by monitoring the absorbance of
DNA at 260 nm as a function of temperature. The DNA melting studies were carried out with calf thymus DNA in the absence and presence of \(8a\) and \(9b\) [1: 5 ratio of \(8a\) and \(9b\) to DNA-c(P)]. The \(T_m\) for calf thymus DNA was 60±5 °C in the absence of compounds, whereas in the presence of parent compounds (\(8a\) and \(9b\)) the \(T_m\) of CT DNA were increased by 5 °C and 3 °C, respectively. These DNA melting experiments supported the interaction of \(8a\) and \(9b\) in to the double helix DNA (Figure 3.13).

**Figure 3.13:** Melting curves of CT-DNA in absence and presence of compounds \(8a/9b\).

3.3.6. *In vitro* antioxidant activities

3.3.6.1. Superoxide anion scavenging activity

In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The antioxidant properties of the synthesized \(8a\) and \(9b\) were verified by their catalytic activities in the dismutation of superoxide radicals. The sulfur containing \(9b\) showed to be active catalysts with a better performance of scavenging ability than \(8a\). The SOD activity of these molecules was compared with standard BHT and BHA. The decrease of absorbance at 570 nm with \(8a\)
and 9b indicates the consumption of superoxide anion in the reaction mixture. Thus the newly synthesized compounds possess strong superoxide radical scavenging activity than BHT and BHA.

![Graph](image)

**Figure 3.14:** Superoxide anion radical scavenging activity of 8a/9b with BHA, and BHT by the PMS-NADH-NBT method (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene).

The results were found statistically significant (p < 0.05). The percentage inhibition of superoxide generated by 60 μg/mL concentration of comps was found to be 65% for 8a and 76% for 9b which is greater than that of BHT (51%) but less than BHA (81%) respectively, at same concentration as shown in **figure 3.14**.

### 3.3.6.2. Scavenging of hydrogen peroxides

Hydrogen peroxide itself is not very reactive, but it can be toxic and sometime, it may give rise to a hydroxyl radical [51]. Thus, removing H₂O₂ is very important for protection of biological systems. Our synthesized compounds are capable of scavenging...
hydrogen peroxide in a concentration-dependent manner. This was determined according to the method [52]. The scavenging ability of compounds on hydrogen peroxide is shown in figure 15. At 60 µg/mL concentration compounds 8a/9b exhibited 68 and 74% scavenging effect over hydrogen peroxide. Thus, statistically these results are significant and followed the order for inhibition: (BHA > 9b > 8a > BHT) for hydrogen peroxide.

![Figure 3.15: Percentage of inhibition plot for 8a/9b against standard BHA, and BHT.](image)

**Figure 3.15:** Percentage of inhibition plot for 8a/9b against standard BHA, and BHT.

### 3.3.6.3. Free radical (DPPH) scavenging activity

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm leading to the activity. The decrease in absorbance of DPPH radical was caused by antioxidants because of the reaction between 8a/9b with DPPH radical progresses and these results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration. Hence, DPPH is an important substrate to evaluate antioxidant activity [53,54]. **Figure 16** illustrates significant (p<0.01) decrease in the concentration of DPPH radical due to the scavenging ability of compounds compared to standards. The results indicate that the sulfur containing 9b
showed stronger DPPH scavenging activity than oxygen containing 8a. Statistically, the scavenging effect of both compounds with DPPH radical decreased in the following order: BHA > 9b > 8a > BHT with 64, 61, 55, and 48% of inhibition, respectively.

![Percentage of inhibition plot](image)

**Figure 3.16:** Percentage of inhibition plot belongs to 8a/9b for DPPH free radical scavenging activity at (20-60 μg/ml) concentration with BHA, and BHT.

3.3.6.4. Reducing power

The reducing capacity of both the compounds may serve as a significant indicator of its potential antioxidant activity [55]. The antioxidant activity of putative antioxidants has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging. The reducing power of 8a/9b increased with decrease in absorbance value. The compound 9b exhibited stronger reducing power than 8a, may be due to the presence of sulfur in that molecule but this difference between the compounds was found to be statistically significant (p>0.06) with standard compounds followed the order: BHA > 9b > 8a > BHT which as shown in the figure 3.17.
3.3.6.5. Hydroxyl radical (HO) scavenging activity

In the case of scavenging effect of hydroxyl radical, we found that the complexes are good scavengers of OH radicals in a concentration-dependent manner. BHT and BHA were used as standard hydroxyl radical scavenging reagent. The activity of standards compared with 8a and 9b indicates that the hydroxyl radical scavenging ability increases with decrease in the absorbance at 520 nm as shown in figure 3.18.

Figure 3.17: Bar graph showing the percentage of inhibition for reducing power of compounds 8a/9b with BHA, and BHT.

Figure 3.18: Percentage inhibition plot for hydroxy radical scavenging activity at (20-60 μg/ml) concentration of 8a/9b, BHA, and BHT by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.
3.4. Conclusion

In conclusion, the author developed an simple and efficient method for different scaffold of biologically important quinoline/ benzo[h]quinoline -3-carbonitriles and 2H-pyrano/2H-thiopyrano[2,3-b]quinoline-2-carboxylic acids (8a/9b) using nanostructured TiO$_2$ catalyst based on microwave irradiation technique. The results supported that the new molecule bound to DNA via intercalative modes and also they are strong antioxidants. They protect DNA from oxidative DNA damage caused by free radicals. Therefore, it is suggested that further work could be performed on similar quinoline analogues as antioxidant compounds to evaluate their *vivo* effects.
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