Chapter -7

Utility of Aqueous Mediated Synthesis, and DNA Binding Interaction Behavior of Novel Fused-quinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1H)thione/one derivatives

Journal of Nucleosides, Nucleotides and Nucleic Acids (in press)
2009
Introduction

Chalcones are a class of privileged structures that have a wide range of biological properties [1]. Chalcones are reported as anticancer agents [2], and antimalarial agents [3,6]. Quinoline-based fused heterocyclic systems are found as potential anticancer agents [7] and have antimalarial activities [8]. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological pharmaceutical and therapeutical activities [9]. The literature reported some of quinolinyl chalcones and pyrimidines were used as antimicrobial agents.

The pyridine ring is one of the most well-known systems among the naturally occurring heterocycles [10]. Pyridine and fused pyridine moieties present in numerous natural products such as quinoline and isoquinoline alkaloids, [11] and nicotine and its analogues [12]. 2- Aminopyridines are promising substituted pyridines which have been shown to be biologically active molecules [13]. Additionally, because of their chelating
abilities, 2-aminopyridines are commonly used as ligands in inorganic and organometallic chemistry [14]. If substituted with optically active groups, they could potentially serve as chiral auxiliaries or chiral ligands in asymmetric reactions. For these reasons, 2-aminopyridines are valuable synthetic targets. The synthesis of 2-aminopyridine derivatives has been extensively reviewed [15-18].

![Chemical structures](image)

Recently, the heterocycles are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics, etc. [19]. Hence, they have attracted considerable attention in the design of biologically active molecules [20]. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry. Among the heterocycles, 1,3-thiazines are a class of compounds with biological activity, such as antimicrobial, antitumor, antioxidant, calcium channel modulators, and antipyretic [21,23]. On the other hand, the classes of pyrimidines possess a broad spectrum of biological effectiveness such as antitubercular, calcium channel blockers [24], and many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use. Apart from these, chalcones have been reported to possess various biological activities such as antibacterial [25], antitumor [26], anticancer [27], and prostaglandin binding [28] properties.
Present were the chalcones are important starting materials for the synthesis of different classes of heterocyclic compounds such as pyrazolines, thiophenes and pyrimidines. The synthesis of novel pyrimido-quinoline derivatives by a convenient method remains an important synthetic task, because of their great importance in pharmacological field [29]. Many of these compounds have proved to be active anticancers, anti-inflammatories, antiallergics and antimicrobials antimalarial [30] drugs have attracted chemists all over the world to prepare a new series of derivatives for their biological activities [31].

Present work

The important of the quinoline containing dihydropyrimidine we attempt to prepare 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives, and synthesized compounds were interact with CT-DNA.
Experimental section

The chemicals used for the synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives were of analytical grade. The instruments used for structural elucidation is presented in chapter-2.

UV-Visible spectroscopic

The DNA binding experimental part is discussed in the chapter-2.

General procedure

Synthesis of (2)-1-(2-hydroxyquinolin-3-yl)-3-phenylprop-2-en-1-one (3)

To a mixture of ketone (2) (1.63 ml 0.014 mol) and the appropriate aldehyde (1) (2.55 g 0.014 mol) in oxygen-free ethanol (25) was added a solution of sodium hydroxide in distilled water (5 ml) with constant stirring at 0°C. The reaction mixture was stirred at 60 ± 5 °C for 2hr on magnetic stirrer. The completion of reaction was checked by TLC and poured in ice-cold water. The solid mass that separated out was filtered, washed with water and crystallized from ethyl acetate to furnish the desired product as yellowish crystals. Yield 79% 180-182°C.

Synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1H)-thione(2a)

A mixture of (1.55 g 0.005 mol) required chalcone (3), (0.42 g 0.0056 mol) thiourea and KOH (0.66 g) in 20 ml ethanol was heated under reflux for 6-8 hr. The progress of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice greenish yellow solid product was separated by neutralizing dil HCl and recrystalized from ethanol Yield 83 %.
Results and discussion

The strategy adapted for the recent interest in green chemistry has focused a new challenge for organic synthesis to find new reaction conditions which reduce the emission of volatile organic solvents and the use of hazardous toxic chemicals. Organic reactions in aqueous media have attracted increasing interest currently because of environmental issues and the understanding of biochemical processes. Water as reaction solvent, offers many practical and economic advantages including low cost, safe handling and environmental compatibility.

The IR spectra of the compound (2a-f) showed two broad peaks in the region of 3424 cm\(^{-1}\) 3209 cm\(^{-1}\) due to \(-\text{OH}\)- and \(-\text{NH}\)- groups, respectively. The generation of new sharp absorption band at 2696 cm\(^{-1}\) attributed to tautomeric form of (SH) stretching frequency \([32, 33]\) Further, the structure (2a) was confirmed by \(^1\text{H}-\text{NMR}\) spectra which showed a broad peak at \(\delta: 11.18-11.08\) due to (Ar-OH-) second position of quinoline (2a) and resonate peak \(\delta: 11.32-11.23\) ppm, corresponds to the cyclized (-NH-) pyrimedene. The signal exhibits multiplets at \(\delta: 7.18-8.45\) ppm for aromatic protons, \([34, 35]\) and its mass spectra having molecular ion peak at \(m/z =334[M^+]\). The obtained elemental analysis values are in good agreement with theoretical data. The synthesized six more title compounds, which exhibited similar spectral data, which are summarized in Table-7.1 and 7. 2.
Scheme-7.1. Synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives
Table-7.1. Physical and analytical data of synthesized 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives (2a-f).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Color</th>
<th>Yield %</th>
<th>M.p °C</th>
<th>Cryst solvent</th>
<th>Molecular Formula</th>
<th>Analysis Calcd (Found) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mol/wt</td>
<td>C</td>
</tr>
<tr>
<td>2a</td>
<td>Yellowish</td>
<td>83</td>
<td>215-217</td>
<td>Ethanol</td>
<td>C_{19}H_{13}N_{3}O_{2}S_{2} (334.80)</td>
<td>68.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(68.47) (4.44) (12.56) (9.68)</td>
</tr>
<tr>
<td>2b</td>
<td>Yellowish</td>
<td>78</td>
<td>250-252</td>
<td>Ethyl acetate</td>
<td>C_{20}H_{17}N_{3}O_{2}S_{2} (346.83)</td>
<td>69.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(69.17) (4.94) (12.02) (9.28)</td>
</tr>
<tr>
<td>2c</td>
<td>Yellowish</td>
<td>90</td>
<td>238-240</td>
<td>Ethyl acetate</td>
<td>C_{20}H_{17}N_{3}O_{2}S_{2} (364.83)</td>
<td>66.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(66.17) (4.74) (11.51) (8.88)</td>
</tr>
<tr>
<td>2d</td>
<td>Brown</td>
<td>93</td>
<td>225-226</td>
<td>Ethanol</td>
<td>C_{19}H_{13}N_{3}O_{2} (318.81)</td>
<td>71.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(71.96) (4.69) (13.29)</td>
</tr>
<tr>
<td>2e</td>
<td>Dark brown</td>
<td>90</td>
<td>246-248</td>
<td>Ethanol</td>
<td>C_{20}H_{17}N_{3}O_{2}S_{2} (331.84)</td>
<td>72.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(72.37) (5.20) (12.66)</td>
</tr>
<tr>
<td>2f</td>
<td>Brown</td>
<td>93</td>
<td>240-242</td>
<td>Ethanol</td>
<td>C_{20}H_{17}N_{3}O_{3} (348.14)</td>
<td>69.15</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
<td>(69.20) (4.91) (12.06)</td>
</tr>
</tbody>
</table>

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Table 7.2. IR, $^1$H-NMR and mass characterization of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives (2a-f).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>IR cm$^{-1}$ (KBr)</th>
<th>$^1$H-NMR, (CDCl$_3$)δ : (ppm) (400 MHz)</th>
<th>Mass, m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>3430 (OH-quinoline), 3209 (-NH pyrimidine), 3003, 2926 (Ar-CH stretching), 1587 (C=N), 2696 (SH), 1499 (C=C pyrimidine moiety)</td>
<td>13.16 (s, 1H, OH quinoline), 11.32 (s, 1H, NH pyrimidine), 7.26-7.53 (m, 5H, 4H, 5H, 6H, 7H, and 8H, Ar-H quinoline). 3.67 (d, 1H, -CH$_2$), 4.23 (d, -CH$_3$), 7.85-8.43 (m, 5H, Ar-H, phenyl).</td>
<td>334[M$^+$.]</td>
</tr>
<tr>
<td>2b</td>
<td>3430 (OH-quinoline), 3210 (-NH pyrimidine), 3006, 2924 (Ar-CH stretching), 1588 (C=N), 2696 (SH), 1497 (C=C pyrimidine moiety)</td>
<td>13.15 (s, 1H, OH Quinoline), 11.31 (s, 1H, NH pyrimidine), 3.22, (s, 3H, CH$_3$), 3.64 (d, 1H, -CH$_2$), 4.22 (d, 2H, -CH$_2$), 7.15-7.53 (m, 4H, 4H, 5H, 7H, and 8H, Ar-H quinoline).</td>
<td>347[M$^+$.]</td>
</tr>
<tr>
<td>2c</td>
<td>3432 (OH-quinoline), 3207 (-NH pyrimidine), 3002, 2928 (Ar-CH stretching), 1589 (C=N), 2696 (SH), 1496 (C=C pyrimidine moiety), 1687 (C=O), 3424 (OH-quinoline), 3109 (-NH pyrimidine), 2926, (Ar-CH stretching), 1576 (C=N), 1414 (C=C pyrimidine moiety)</td>
<td>13.16 (s, 1H, OH quinoline), 11.33 (s, 1H, NH pyrimidine), 3.86 (s, 3H, -OCH$_3$), 3.64 (d, 1H, -CH$_2$), 4.20 (d, 2H, -CH$_2$), 7.15-7.53 (m, 4H, 4H, 5H, 7H, and 8H, Ar-H quinoline).</td>
<td>363 [M$^+$.]</td>
</tr>
<tr>
<td>2d</td>
<td>1687 (C=O), 3424 (OH-quinoline), 3109 (-NH pyrimidine), 2926, (Ar-CH stretching), 1576 (C=N), 1414 (C=C pyrimidine moiety)</td>
<td>13.10 (s, 1H, OH quinoline), 11.08 (s, 1H, NH pyrimidine), 7.31-7.55 (m, 5H, 4H, 5H, 6H, 7H, and 8H, Ar-H quinoline). 3.63 (d, 1H, -CH$_2$), 4.21 (d, 2H, -CH$_2$), 7.62-8.23 (m, 5H, Ar-H, phenyl).</td>
<td>318 [M+H].</td>
</tr>
<tr>
<td>2e</td>
<td>1689 (C=O), 3422 (OH-quinoline), 3110 (-NH pyrimidine), 2926, (Ar-CH stretching), 1576 (C=N), 1414 (C=C pyrimidine moiety)</td>
<td>13.12 (s, 1H, OH quinoline), 11.09 (s, 1H, NH pyrimidine), 3.20 (s, 3H, CH$_3$), 3.66 (d, 1H, -CH$_2$), 4.20 (d, 2H, -CH$_2$), 7.38-7.53 (m, 5H, 4H, 5H, 7H, and 8H, Ar-H quinoline).</td>
<td>331[M$^+$.]</td>
</tr>
<tr>
<td>3f</td>
<td>1688 (C=O), 3425 (OH-quinoline), 3110 (-NH pyrimidine), 2928, (Ar-CH stretching), 1575 (C=N), 1414 (C=C pyrimidine moiety)</td>
<td>13.12 (s, 1H, OH quinoline), 11.09 (s, 1H, NH pyrimidine), 3.82 (s, 3H, -OCH$_3$), 3.66 (d, 1H, -CH$_2$), 4.19 (d, 2H, -CH$_2$), 7.38-7.53 (m, 5H, 4H, 5H, 7H, and 8H, Ar-H quinoline).</td>
<td>347[M$^+$.]</td>
</tr>
</tbody>
</table>
Fig-7.1. IR spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5, 6-dihydropyrimidine-2(1H)-thione(2a)
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Fig-7.2. $^1$HNMR spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1H)-thione (2a).
Fig-7.3. Mass spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1H)-thione (2a).

Calcd M/z = 333.84, Found, 334.1
Fig-7.4. IR spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-one (2d)
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![NMR spectrum](image_url)

Fig-7.5. $^1$H-NMR spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-one(2d)
Fig-7.6. Mass spectra of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-one (2d)
DNA interaction studies (absorption spectral studies)

The binding of the molecules to DNA has been well characterized by the large hypochromism. After intercalation the $\pi^*$ orbital of compounds could couple with $\pi$ orbital of base pairs, thus decreasing the $\pi^* \rightarrow \pi$ transition of energy, and resulting bathochromism. Hence the decrease the absorption intensity and significant red shift due to stacking interaction between drug and CT-DNA. [36, 37] In the present studies the DNA binding studies were characterized by absorbance maximum at 329 nm for (2a) and 259 nm for (2d). The addition of increasing higher concentration of DNA led to hypochromic and bathochromic (red shift) changes in its visible absorption spectra as a result of formation of more stable complexes (Figure-7.7, 7.8). The interaction of (2a) and (2d) with CT-DNA resulted in the decrease of absorption intensity accompanied by a shift towards higher wavelengths (~3 and 5 nm). Around 12% to 9% reduction intensity of absorption was observed at 329 and 259 nm peak maxima in the presence of an excess of calf thymus DNA. [38] The lowest observation value observed in spectral changes (including red shift, hypochromicity) were used to evaluate intrinsic binding constant ($K_b$), it observed value $4.3 \times 10^5 \text{ M}^{-1}$ for (2a) and $3.8 \times 10^5 \text{ M}^{-1}$ for (2d) from the spectral result suggested that compound (2a) bind more strongly with base pairs than that of (2d) [39-42].
Fig-7.7. UV- absorption spectra in Tris-HCl Buffer upon addition CT- DNA (2a) [DNA]=0.5μm, =10μm, drug, 20μm; 30μm; 40μm; 50μm; Arrow shows the absorbance changing upon the increase of DNA concentration.
**Fig-7.8.** UV- absorption spectra in Tris-HCl Buffer upon addition CT- DNA (2d) [DNA]=0.5μm, =10μm, drug, 20μm; 30μm; 40μm; 50μm; Arrow shows the absorbance changing upon the increase of DNA concentration.
Viscosity measurements

To further clarify the interaction modes of (2a) and (2d) with DNA were investigated by viscosity measurements. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process. [43, 44] The viscosity result shows that the viscosity changes in short, rod-like DNA fragments. The relative length increase \( \frac{L}{L_0} \) of the complex formed between (2a), (2d) and DNA is shown in (Figure-7.9). It is evident that binding of (2a) and (2d) increased the viscosity of DNA corresponding to an increase in the contour length of the DNA fragments. Hence the presence of compound had an obvious effect on relative viscosity of CT-DNA with an increase in concentration of the added compounds. [45, 47]

![Fig-7.9. Effect of increasing amount of the (2a) and (2d) on the relative viscosities of CT-DNA, at 25°C.](image)

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Thermal denaturation studies

Other strong evidence for the intercalative binding of (2a) and (2d) 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. [48-51] The molar extinction coefficient of DNA bases at 260 nm in the double helical form is much less than the single stranded form; hence, melting of the helix leads to an increase in the absorbance at 260 nm. The DNA melting studies were carried out with calf thymus DNA in the absence and presence of (2a) and (2d). \(T_m\) (melting temperature) for calf thymus DNA was 60 ± 5 °C in the absence of compounds, but in the presence of (2a) and (2d) the \(T_m\) of CT-DNA increased by 7 to 10°C. These variations in DNA melting temperature strongly supported the intercalation of compounds into the double helix DNA (Fig-7.10).

![Fig-7.10. Melting curves of CT-DNA in the presence and absences of (2a) and (2d).](image-url)
Conclusions

In conclusion the synthetic approach adopted for synthesis of pyrimidenequinoline derivatives (2a-f) is ecofrindly. In DNA binding studies, indicate that increasing DNA concentration leads to hypochromicity and bathochromic shifts of the compounds (2a) and (3a). The binding constant values of $4.3 \times 10^5 \text{ M}^{-1}$ for (2a) and $3.8 \times 10^5 \text{ M}^{-1}$ for (2d) suggested that the compound (2a) bind more strongly to CT-DNA than (2d). In addition, increasing viscosity of sonicated rod-like DNA fragments and the melting temperature of CT-DNA, in the presence of compound solutions supports the binding mode.
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