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Solvent-Free Solid Phase Syntheses of 2-Chloroquinoline-3-carbaldehyde Phenyl Hydrazones and their DNA Cleavage Studies

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ABSTRACT

In this article, the authors describe the synthesis of 2-chloroquinoline-3-carbaldehyde phenyl hydrazones by two methods – (1) in solution, by stirring the reactants in MeOH at room temperature over a long period of time (2-15 h) and, (2) in solid state by grinding reactants together to form products in dramatically short time (in <15 min). The hydrazones obtained are tested for their DNA cleavage properties and some of them are found to show good chemical nuclease activity in the presence of both oxidizing agent (H_2O_2) and reducing agent (MPA). Some of them exhibited hydrolytic activity, and their antioxidant activity was found to be very low.

Keywords: Solvent free synthesis, Green chemistry, DNA cleavage, Quinoline, Hydrazone.

INTRODUCTION

Hydrazones are an important class of organic compounds as they possess various bioactivities such as antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, and antitumoral activities [1]. The heterocyclic hydrazones have attracted attention of medicinal chemists due to their wide ranging pharmacological properties including iron scavenging and antitubercular activities [2]. Hydrazones are essential intermediates in the synthesis of several heterocyclic compounds [3-5]. For instance, phenyl hydrazones are intermediates in the Fischer synthesis of indoles [6, 7], 1*H*-indazoles [8], pyrazoles [9] and pyrazoloquinolines etc [10-14]. In view of such a wide range of applications of hydrazones and substituted hydrazones, a number of researchers have expended their efforts to synthesize and to study their biological activities and other uses. The synthetic procedures have usually involved solvents, acid or base catalysts, temperatures higher than room temperature and long reaction times that may run to several hours [15-19]. In order to minimize the adverse environmental consequences caused by using such procedures [20-23], chemists have been paying much attention to avoid the use of energy, solvent, catalysts and chemicals that do not become part of the products.

We have synthesized substituted quinoline-3-carbaldehyde phenyl hydrazones by grinding [24-30] the two reactants together in solid phase to form, within a few minutes, the hydrazones **3a-k**. As a part of studying their biological function we have carried out the DNA cleavage activity. We report here the result of our solid phase synthesis of the hydrazones **3a-k** and their DNA cleavage studies.

MATERIALS AND METHODS

Melting points were recorded in open capillary and are uncorrected. Chemicals were obtained from SD-fine, Sigma-Aldrich companies and were used without further purification. The CHN analysis recorded in Elementar Vario MICRO cube. ESI-MS spectra were measured by using 5mM ammonium acetate: acetonitrile (95:5 mixture) and 5mM acetonitrile: ammonium acetate (5:95 mixture) as mobile phases A and B respectively by using Open Lynx ESI-LCMS spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in *d*6-DMSO using Bruker AV-400 spectrometers and TMS as an internal reference. The reactions were monitored by using TLC. The purity of the synthesized compounds was verified by TLC and further purification was achieved through recrystallization process using methanol as solvent. The DNA cleavage study was performed by using agarose gel electrophoresis method.

Synthesis of 2-chloroquinoline-3-carbaldehyde phenyl hydrazones (3a-k): The Two Typical Procedures (Method A and B) for the Synthesis of 2-Chloroquinoline-3-Carbaldehyde Phenyl Hydrazone (**3a**) is Described as an Example:

Method A (With MeOH as solvent)*:* A solution of 100 mg (0.520 mmol) of 2-chloroquinoline-3-carbaldeyde **1a** and 75.5 mg (0.520 mmol) of phenylhydrazine hydrochloride **2a** in 5 ml of dried MeOH was stirred at room temperature. The product precipitated gradually, and the reaction was monitored by TLC for the disappearance of the reactants, which took about 15 h. After the reaction is completed the solution was diluted with 5 ml of ice cold water with stirring. The precipitated yellow solid was collected by filtration, washed with water and recrystallized from 20 ml of MeOH to get 133 mg (0.471 mmol, 90%) of pure yellow solid **3a**. The other phenyl hydrazones **3b-k** were prepared following the same procedure (Table 1).

Method B (Without solvent by Grinding)*:* A mixture of 100 mg (0.520 mmol) of 2-chloroquinoline-3-carbaldehyde **1a** and 75.5 mg (0.520 mmol) of phenylhydrazine hydrochloride **2a** is thoroughly ground in a clean and dry mortar for about 15 minutes. Examination of the TLC of the product every five minutes indicated the completion of the reaction in less than 15 min. Then product was recrystallized from MeOH to get 144 mg (0.510 mmol, 98%) of pure yellow solid **3a**. Similarly, the other phenyl hydrazones **3b-k** were prepared.

Selected spectral Data for the compounds 3a-k

(E)-1-((2-Chloroquinolin-3-yl)methylene)-2-phenylhydrazine (3a) : Yellow solid, m.p.; 175^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 6.92-6.94 (m, 1H, Ar), 7.19 (d, *J* = 7.6 Hz, 2H, Ar), 7.31 (d, *J* = 7.6 Hz, 2H, Ar), 7.35 (d, *J* = 8.8 Hz, 1H, -NH), 7.55-7.55 (m, 1H, q), 7.68-7.68 (m, 1H, -CH), 7.88 (d, *J* = 8.0 Hz, 1H, q), 8.02 (d, *J* = 8.4 Hz, 1H, q), 8.14 (s, 1H, q), 8.76 (s, 1H, q) ppm; ^{13}C-NMR (100MHz, DMSO d_6): δ = 112.3 (Ar-C2&6), 119.6 (Ar-C4), 122.0 (q-C2), 127.2 (q-C4), 127.5 (q-C6), 128.3 (q-C8), 129.1 (q-C5), 130.4 (Ar-C3&5), 130.5 (q-C7), 130.9 (q-C3), 133.0 (C10-imine), 144.5 (Ar-C1), 146.0 (q-C9), 147.7 (q-C1) ppm; m/z 282 [M^{+}]. Anal. calcd. for $C_{16}H_{12}ClN_3$: C, 68.21, H, 4.29, N, 14.91; Found: C, 68.04, H, 4.04, N, 14.75.

(E)-1-((2-Chloroquinolin-3-yl)methylene)-2-(3-fluorophenyl)hydrazine (3b) : Yellow shiny solid, m.p. 199^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 6.59-6.60 (m, 1H, Ar), 6.90 (d, *J* = 8.0 Hz, 1H, Ar), 7.02-7.02 (m, 1H, Ar), 7.25-7.27 (m, 1H, Ar), 7.64-7.64 (m, 1H,-NH), 7.76-7.77 (m, 1H, q), 7.93 (d, *J* = 8.4 Hz, 1H, -CH), 8.17 (d, *J* = 8.1 Hz, 1H, q), 8.26 (s, 1H, q), 8.98 (s, 1H, q), 11.14 (s, 1H, q) ppm; ^{13}C-NMR (100

MHz, DMSO-d6): δ = 99.1(Ar-C2), 105.8 (Ar-C6), 108.5 (Ar-C4), 127.2 (q-C2), 127.5 (q-C4), 127.5 (q-C5), 128.4 (q-C8), 130.7 (q-C6), 130.8 (q-C7), 131.9 (Ar-C5), 133.7 (q-C3), 146.2 (C10-imine), 146.5 (Ar-C1), 146.6 (q-C9), 147.8 (q-C1), 162.1 (Ar-C3), 164.5 (Ar-C3) ppm; m/z 300 [M^+]. Anal. calcd. for $C_{16}H_{11}ClFN_3$: C, 64.11, H, 3.70, N, 14.02; Found: C, 63.96, H, 3.92, N, 13.97.

(E)-1-((2-Chloroquinolin-3-yl)methylene)-2-(3,5-difluorophenyl)hydrazine (3c) : Yellow solid, m.p. 205^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 6.54-6.56 (m, 1H, Ar), 6.80 (dd, *J* = 2.8 Hz, 12.0 Hz, 2H, Ar), 7.65 (dd, *J* =1.4 Hz, 10.6 Hz, 1H, -NH), 7.76-7.77 (m, 1H, q), 7.93 (d, *J* = 11.0 Hz, 1H, -CH), 8.17 (d, *J* = 10.4 Hz, 1H, q), 8.27 (s, 1H, q), 9.03 (s, 1H, q), 11.28 (s, 1H, q), ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 94.1 (Ar-C4), 94.4 (Ar-C4), 95.2 (Ar-C2&6), 95.4 (Ar-C2&6), 126.8 (q-C2), 127.2 (q-C4), 127.5 (q-C6), 127.6 (q-C6), 128.5 (q-C8), 130.9 (q-C5), 133.2 (q-C7), 134.2 (q-C3), 146.3 (C10-imine), 147.1 (Ar-C1), 147.3 (Ar-C1), 147.4 (Ar-C1), 147.8 (q-C1), 162.1 (Ar-C3), 162.2 (Ar-C3), 164.5 (Ar-C5), 164.7 (Ar-C5) ppm; m/z 318 [M^+]. Anal. calcd. for $C_{16}H_{10}ClF_2N_3$: C, 60.48, H, 3.17, N, 13.23; Found: C, 60.33, H, 3.02, N, 12.93%.

(E)-1-((2-Chloroquinolin-3-yl)methylene)-2-p-tolylhydrazine (3d) : Orange shiny solid, m.p. 183-185^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 3.32 (s, 3H, Me), 6.83 (t, *J* = 7.2 Hz, 1H, -NH), 7.17 (d, *J* = 8.1 Hz, 2H, Ar), 7.27 (t, *J* = 7.8 Hz, 2H, Ar), 7.61 (d, *J* = 8.5 Hz, 1H, q), 7.82 (d, *J* = 8.4 Hz, 1H, -CH), 7.91 (s, 1H, q), 8.23 (s, 1H, q), 8.80 (s, 1H, q), 10.94 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 20.2 (Ar-Me), 112.4 (Ar-C2&6), 112.7 (q-C2), 127.2 (q-C4), 127.5 (q-C6), 127.6 (q-C8), 128.1 (q-C5), 128.3 (Ar-C4), 129.5 (Ar-C3&5), 129.8 (q-C7), 130.3 (q-C3), 132.8 (Ar-C1), 142.2 (C10-imine), 145.9 (q-C9), 147.7 (q-C1) ppm; m/z 296 [M^+]. Anal. calcd. for $C_{17}H_{14}ClN_3$: C, 69.03, H, 4.77, N, 14.21; Found: C, 68.93, H, 4.52, N, 14.13.

(E)-1-((2-Chloro-6-methylquinolin-3-yl)methylene)-2-phenylhydrazine (3e). Yellow solid, m.p. 173^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 2.32 (s, 3H, Me), 7.10-7.10 (m, 4H, Ar), 7.54-7.58 (m, 1H, Ar), 7.68-7.68 (m, 1H, -NH), 7.89 (d, *J* = 0.8 Hz, 1H, q), 7.98 (d, *J* = 8.8 Hz, 1H, q), 8.02 (s, 1H, -CH), 8.11 (s, *J* = 0.8 Hz, 1H, q), 8.76 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 21.1 (q-Me), 113.4 (Ar-C2&6), 127.8 (q-C2), 127.9 (q-C5), 128.4 (q-C4), 128.6 (q-C8), 130.4 (Ar-C3&5), 130.8 (q-C7), 130.9 (q-C6), 131.5 (q-C3), 134.3 (C10-imine), 142.0 (Ar-C1), 147.2 (q-C9), 148.9 (q-C1) ppm; m/z 296 [M^+]. Anal. calcd. for $C_{17}H_{14}ClN_3$: C, 69.03, H, 4.77, N, 14.21; Found: C, 68.91, H, 4.51, N, 13.97.

(E)-1-((2-Chloro-6-methylquinolin-3-yl)methylene)-2-(3-fluorophenyl)hydrazine (3f) : Yellow crystalline solid, m.p.: 186^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 2.54 (s, 3H, -Me), 6.60-6.60 (m, 1H, Ar), 6.63 (dd, *J* = 2.4 Hz, 8.4Hz, 1H, Ar), 6.81-6.81 (m, 1H, -NH), 6.99 (t, *J* = 2.4 Hz, 1H, Ar), 7.02 (t, *J* = 2.4 Hz, 1H, Ar), 7.21 (s, 1H, q), 7.23 (t, *J* = 1.6 Hz, 1H, -CH), 7.26 (t, *J* = 1.2 Hz, 2H, q), 7.53 (d, *J* = 1.6 Hz, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 21.0 (q-Me), 105.8 (Ar-C2), 108.5 (Ar-C4), 127.0 (Ar-C6), 127.2 (q-C2), 127.2 (q-C5), 130.7 (q-C4), 130.8 (q-C8), 132.0 (Ar-C5), 132.8 (q-C7), 132.9 (q-C6), 137.1 (q-C3), 144.8 (C10-imine), 146.5 (Ar-C1), 146.6 (q-C9), 146.9 (q-C1), 162.1 (Ar-C3), 164.5 (Ar-C3) ppm; m/z 314 [M^+]. Anal. calcd. for $C_{17}H_{13}ClFN_3$: C, 65.08, H, 4.18, N, 13.39; Found: C, 64.79, H, 3.97, N, 13.18.

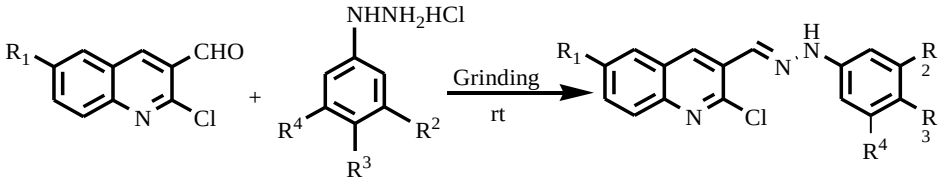
(E)-1-((2-Chloro-6-methylquinolin-3-yl)methylene)-2-(3,5-difluorophenyl)hydrazine (3g) : Yellow solid, m.p.: 220^{o}C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 3.32 (s, 3H, Me), 6.53-6.54 (m, 1H, Ar), 6.78 (dd, *J* = 2.8 Hz, 11.1 Hz, 2H, Ar), 7.62 (dd, *J* = 2.3 Hz, 11.52 Hz, 1H, -NH), 7.82 (d, *J* = 11.4 Hz, 1H, q), 7.92 (s, 1H, q), 8.25 (s, 1H, -CH), 8.91 (s, 1H, q), 11.26 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 21.0 (q-Me), 94.0 (Ar-C4), 94.3 (Ar-C4), 95.1 (Ar-C2&6), 95.4 (Ar-C2&6), 126.6 (q-C2), 127.1 (q-C5), 127.1 (q-C4), 127.2 (q-C8), 132.9 (q-C7), 133.2 (q-C7), 133.4 (q-C6), 137.1 (q-C3), 145.0 (C10-imine), 146.9 (Ar-C1), 147.1 (Ar-C1), 147.3 (q-C9), 147.4 (q-C1), 162.1 (Ar-C3), 162.2 (Ar-C3), 164.5 (Ar-C5), 164.6 (Ar-C5) ppm; m/z 332 [M^+]. Anal. calcd. for $C_{17}H_{12}ClF_2N_3$: C, 61.55, H, 3.65, N, 12.67; Found: C, 61.44, H, 3.35, N, 12.49.

(E)-1-((2-Chloro-6-methoxyquinolin-3-yl)methylene)-2-phenylhydrazine (3h) : Yellow solid, m.p.: 178°C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 3.92 (s, 3H, OCH_3), 6.84 (t, *J* = 7.2 Hz, 1H, q), 7.18 (d, *J* = 7.6 Hz, 2H, Ar), 7.28 (t, *J* = 7.2 Hz, 2H, Ar), 7.41 (dd, *J* = 2.8 Hz, 9.2 Hz, 1H, -NH), 7.59 (d, *J* = 2.8 Hz, 1H, Ar), 7.83 (d, *J* = 9.2 Hz, 1H, q), 8.22 (s, 1H, -CH), 8.84 (s, 1H, q), 10.97 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 55.6 (q-Me), 106.0 (q-C5), 112.3 (Ar-C2&6), 119.6 (Ar-C4), 123.0 (q-C7), 127.5 (q-C2), 128.5 (q-C4), 128.9 (q-C8), 129.1 (Ar-C3&5), 130.6 (q-C3), 131.9 (C10-imine), 142.1 (Ar-C1), 144.5 (q-C9), 145.1 (q-C1), 157.8 (q-C6) ppm; m/z 310 [M^-]. Anal. calcd. for $C_{17}H_{14}ClN_3O$: C, 65.49, H, 4.53, N, 13.48; Found: C, 65.23, H, 4.21, N, 13.13.

(E)-1-((2-Chloro-6-methoxyquinolin-3-yl)methylene)-2-(3-fluorophenyl)hydrazine (3i) : Yellow solid, m.p.: 187-189°C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 2.47 (s, 3H, -OMe), 6.59 (d, *J* = 8.9 Hz, 1H, Ar), 6.91 (d, *J* = 10.4 Hz, 1H, Ar), 7.03 (s, 1H, Ar), 7.27 (t, *J* = 9.4 Hz, 1H, q), 7.59 (d, *J* = 11.4 Hz, 1H, Ar), 7.79 (d, *J* = 11.4 Hz, 1H, -NH), 7.88 (s, 1H, -CH), 8.21 (s, 1H, q), 8.82 (s, 1H, q), 11.09 (s, 1H, q), ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 55.6 (q-OMe), 105.8 (Ar-C2), 106.1 (Ar-C4), 108.5 (q-C5), 123.2 (Ar-C6), 127.1 (q-C7), 128.4 (q-C2), 128.9 (q-C4), 130.7 (q-C8), 130.8 (q-C8), 132.0 (Ar-C5), 132.4 (Ar-C5), 142.3 (q-C3), 142.3 (q-C3), 145.2 (C10-imine), 145.2 (Ar-C1), 146.5 (q-C9), 146.6 (q-C1), 157.9 (q-C6), 162.1 (Ar-C3), 164.5 (Ar-C3) ppm; m/z 330 [M^+]. Anal. calcd. for $C_{17}H_{13}ClFN_3O$: C, 61.92, H, 3.97, N, 12.74; Found: C, 61.70, H, 3.93, N, 12.64.

(E)-1-((2-Chloro-6-methoxyquinolin-3-yl)methylene)-2-p-tolylhydrazine (3j) : Yellow solid, m.p.: 171°C, ^{1}H-NMR (400 MHz, DMSO-d6): δ = 2.53 (s, 3H, Me), 3.31 (s, 3H, OMe), 7.42 (d, *J* = 8.4 Hz, 3H, Ar), 7.56 (d, *J* = 7.4 Hz, 2H, q&Ar), 7.70 (t, *J* = 7.36 Hz, 1H, -NH), 7.85 (dd, *J* = 0.8 Hz, 11.3 Hz, 1H, q), 7.98 (d, *J* = 8.4 Hz, 1H, -CH), 8.22 (d, *J* = 8.0 Hz, 1H, q), 9.09 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ = 21.0 (Ar-Me), 53.6 (q-OMe), 115.3 (q-C5), 118.0 (Ar-C8), 125.5 (Ar-C2), 127.2 (q-C7), 127.4 (q-C2), 127.9 (q-C4), 128.6 (Ar-C4), 129.6 (q-C8), 129.9 (Ar-C5), 131.7 (Ar-C3), 133.3 (q-C3), 135.0 (Ar-C1), 135.9 (C10-imine), 139.2 (q-C9), 140.5 (q-C1), 142.0 (q-C6) ppm; m/z 326 [M^+]. Anal. calcd. for $C_{18}H_{16}ClN_3O$: C, 66.36, H, 4.95, N, 12.90; Found: C, 66.07, H, 4.69, N, 12.82.

(E)-1-((2-Chloroquinolin-3-yl)methylene)-2-(3-nitrophenyl)hydrazine (3k) : Yellow solid, m.p.: 188°C,^{1}H-NMR (400 MHz, DMSO-d6): δ = 7.09 (s, 4H, -NH &Ar), 7.65 (t, *J* = 6.8 Hz, 1H, q), 7.75-7.75 (m, 1H, -CH), 7.92 (d, *J* = 8.36 Hz, 1H, Ar), 8.15 (d, *J* = 8.04 Hz, 1H, q), 8.21 (s, 1H, q), 8.90 (s, 1H, q), 10.89 (s, 1H, q) ppm; ^{13}C-NMR (100 MHz, DMSO-d6): δ =112.1 (Ar-C4), 112.3 (Ar-C2&C6), 119.6 (q-C2), 122.0 (q-C4), 127.2 (q-C6), 127.5 (q-C7), 128.3 (q-C5), 129.1 (Ar-C5), 130.4 (q-C7), 130.5 (q-C3), 130.9 (C10-imine), 133.0 (Ar-C1), 144.5 (Ar-C3), 146.0 (q-C8), 147.7 (q-C1) ppm; m/z 325 [M^-]. Anal. calcd. for $C_{16}H_{11}ClN_4O_2$: C, 58.82, H, 3.39, N, 17.15; Found: C, 58.58, H, 3.08, N, 16.99.



1a-c **2a-e** **3a-k**

1a: R^1 = H
1b: R^1 = CH_3
1c: R^1 = OCH_3

2a: R^2 = H, R^3= H, R^4 = H
2b:R^2 = F, R^3= H, R^4 = H
2c: R^2 = F, R^3= H, R^4 = F
2d: R^2 = H, R^3= CH_3, R^4 = H
2e: R^2 = NO_2, R^3 = H, R^4 = H

Scheme 1. General synthetic procedures for the synthesis of 2-chloroquinoline-3-carbaldehyde phenyl hydrazones **(3a-k)**

RESULTS AND DISCUSSION

Though aryl hydrazones of aryl aldehydes, known as Schiff bases, are well known, the study of any hydrazones (**3**) of 2-chlororquinoline-3-carbaldehydes (**1**) is scanty. Srivastava *et al*, have synthesized the phenyl hydrazone **3a** in 50% yield, by refluxing a solution of **1a** and **2a** in methanol [31]. To our knowledge this is the only report of synthesis of Schiff base of **1a**. However, 2-chloroquinoline-3-carbaldehyde hydrazones (**3a-c**) are known to form as intermediates in the synthesis of corresponding pyrazolo[3,4-*b*]quinolines from **1a** and **2a-c** [1-5]. It is obvious that these useful hydrazones [1-14], whether used as end products or formed as intermediates, need improved method for their synthesis.

Our endeavour was to develop mild and environmentally beneficial conditions for their synthesis. Initially, we stirred the methanolic solutions of **1a-c** and **2a-e** at room temperature and allowing the reactions to run as long as it was necessary to complete them, their progress being monitored by TLC. The reaction took 2-15h depending on the degree of substitution on phenyl hydrazine hydrochloride (**2a-e**). The yields are excellent.

Table 1. Solvent-free synthesis of 2-chloroquinoline-3-carbaldehyde phenyl hydrazones (**3a-k)** from 2-chloro-3-formyl quinolines and phenylhydrazine hydrochlorides

En-try	Pro-duct	R^1	R^2	R^3	R^4	In MeOH at rt		Solvent free at rt	
						Time (h)	Yield (%)	Time (min)	Yield (%)
1	**3a**	H	H	H	H	15	90	15	98
2	**3b**	H	F	H	H	4	92	15	97
3	**3c**	H	F	H	F	2	94	15	99
4	**3d**	H	H	CH_3	H	4	90	15	97
5	**3e**	CH_3	H	H	H	15	90	15	97
6	**3f**	CH_3	F	H	H	4	90	15	99
7	**3g**	CH_3	F	H	F	2	94	15	97
8	**3h**	OCH_3	H	H	H	15	88	15	96
9	**3i**	OCH_3	F	H	H	4	90	15	98
10	**3j**	OCH_3	H	CH_3	H	4	93	15	96
11	**3k**	H	NO_2	H	H	4	90	15	95

Following this successful attempt, we thought of avoiding the solvent altogether, to see whether the reaction can be made truly environmental friendly. With this aim, we carried out the condensation of equivalent amount of the two reactants **1** and **2** by grinding them together in solid phase at room temperature with no other solvent or reagent. To our happiness, we found that the reaction went through in less than 15 min giving quantitative yields of the products **3a-k**. We are sure that there can be no simpler and better method for the preparation of 2-chloroquinoline-3-carbaldehyde phenyl hydrazones. The results are included in Table 1. The compounds were characterized by their spectral and melting point data. Since all these compounds are new except **3a**, we wanted to look at their biological activities. As a first study in this direction we have investigated their DNA cleavage.

DNA Cleavage Studies: The extent of cleavage of supercoiled (SC) DNA in the presence of the hydrazones, oxidizing agent H_2O_2 and reducing agent MPA, was determined by agarose gel electrophoresis method. In a typical reaction, supercoiled pUC19 DNA (0.2 µg), taken in 50 mM tris-HCl buffer (pH 7.2) containing 50 mM NaCl, was treated with the hydrazones. The extent of cleavage was measured from the intensities of the bands using UVITEC Gel documentation system. For mechanistic investigations, inhibition reactions were done on adding the reagents prior to the addition of the hydrazones. The solutions were incubated for 1 h in a dark chamber at 37^0 C followed by addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol (2 µL), and the solution was finally loaded on 0.8% agarose gel containing 1.0 µg/ml ethidium bromide (EB). Electrophoresis was carried out for 2 h at 60 V in tris-acetate-EDTA (TAE) buffer. Bands were visualized by UV light and photographed for analysis. Due corrections were made to the observed intensities for the low level of NC form present in

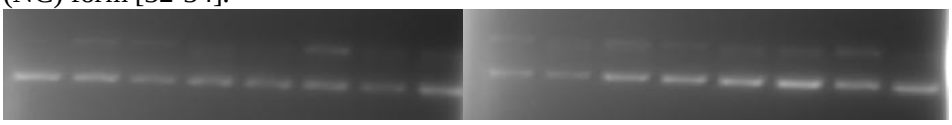
the original sample of SC DNA and for the low affinity of EB binding to SC in comparison to nicked-circular (NC) and linear forms of DNA [32-34].

Table 2 Selected cleavage data of SC pUC19 (0.2 µg, 33.3 µM NP) by 2-chloro quinolone-3- carbaldehyde phenyl hydrazones (**3a-k**).

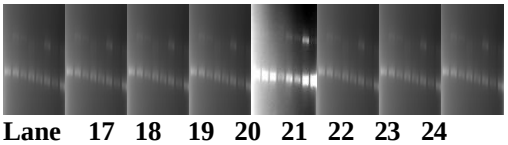
Sl. No.	Reaction Condition	Organic compounds (**3a-k**) (µM)	% NC
1	DNA + Buffer		-
2	DNA + 3a	100	33
3	DNA + 3a+H_2O_2	100	41
4	DNA + 3a+MPA	100	--
5	DNA + 3c	100	--
6	DNA + 3c+ H_2O_2	100	44
7	DNA + 3c+MPA	100	25
8	DNA + 3g	100	--
9	DNA + 3g+ H_2O_2	100	--
10	DNA + 3g+MPA	100	--
11	DNA + 3j	100	43
12	DNA + 3j+ H_2O_2	100	39
13	DNA + 3j+MPA	100	40
14	DNA + 3d	100	--
15	DNA + 3d+ H_2O_2	100	36
16	DNA +3d+MPA	100	--
17	DNA+ 3k	100	--
18	DNA+ 3k+ H_2O_2	100	40
19	DNA+ 3k+ MPA	100	--
20	DNA+ 3h	100	--
21	DNA+ 3h+ H_2O_2	100	57
22	DNA+ 3h+ MPA	100	12
23	DNA+ H_2O_2		
24	DNA + MPA		

[a] $[H_2O_2]$ = 200µM , [b] [MPA] = 5mM

Hydrolytic, oxidative and reductive DNA cleavage activity of **3a-k** have been investigated by agarose gel electrophoresis using supercoiled (SC) plasmid pUC19 DNA (0.2 µg, 33.3 µM NP) in 50mM tris-HCl/50mM NaCl buffer (pH 7.2). Hydrogen peroxide (H_2O_2) (200µM) and 3-mercapto propionic acid (MPA) (5mM) were used as oxidizing and reducing agents respectively. Selected DNA cleavage data are given in Table 2. Hydrazones in 100µM solution showed moderate "cleavage activity" in presence of H_2O_2. 100 µM **3d**, **3h, 3k** Showed the conversion of SC to its nicked-circular form (NC) of DNA in presence of H_2O_2 (oxidative cleavage). 100 µM **3c** Showed cleavage activity in presence of H_2O_2 and MPA (both oxidant and reductant cleavage). 100 µM **3j** Showed oxidative, reductive and hydrolytic cleavage. 100 µM **3a** Showed oxidative and hydrolytic cleavage. Control experiments with H_2O_2, MPA or aryl hydrazones **3a-k** alone, except **3a** and **3j**, do not show any apparent conversion of SC to its nicked-circular (NC) form [32-34].



Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



Lane 17 18 19 20 21 22 23 24

Fig. 1: Gel electrophoresis diagram showing the cleavage of SC pUC19 DNA by different organic compounds (**3a-k**) in 50 mM tris-HCl/50 mM NaCl buffer (pH 7.2) in the presence of oxidizing and reducing agents.

APPLICATIONS

The synthesized hydrazones are tested for their DNA cleavage properties and some of them are found to show good chemical nuclease activity in the presence of both oxidizing (H_2O_2) and reducing (MPA) agents. Some of them exhibited hydrolytic activity, and their antioxidant activity was found to be very low.

CONCLUSIONS

We have effectively developed an efficient green strategy for the synthesis of different 2-chloroquinoline-3-carbaldehyde phenyl hydrazones through grinding technique by using corresponding 2-chloroquinoline-3-carbaldehydes and phenylhydrazine hydrochlorides. All these compounds have been subjected their DNA cleavage studies.

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