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# An Efficient One Pot Synthesis of Biologically Active Quinoline Trifluoro Esters and DNA Studies

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## ABSTRACT

We deliver chemistry of multicomponent reaction (MCR) for 2-Benzyl-5-(2-benzyloxycarbonylamino-3-Phenyl-propylamino)-4-(2-chloro-quinoline-3yl)-5-(2,2,2-trifluoro acetoxy)-pentanoic acid methyl ester (**6a-g**) by flexible Ugi multi-component approach, through commercially available amino acid, aldehydes, isocyanides and amino acid ester in one pot. Further, the binding activity with CT DNA and nucleus property with pUC 19 DNA were studied by absorption spectra ($K_b$ constant is= $4.2 \times 10^{-4}$ $M^{-1}$). The DNA binding results explored that the new molecule bind to DNA through interaction and also an efficient nucleus agent.

**Keywords:** Multicomponent synthesis, Ugi reaction, Quinolines, DNA Binding, Photonuclease activity.

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# INTRODUCTION

Recent development in combinatorial chemistry made remarkable milestone as a tool for drug discovery [1, 2]. Traditional approaches which involve separate synthesis of each individual analog or desired compound, combinatorial synthesis aims to yield large numbers of compounds based on a common core structure with a minimum of time and effort. The first MCRs were accomplished in 1838 when Laurent and Gerhardt formed the “benzoylazotide” from bitter almond oil and ammonia *via* benzaldehyde, hydrogen cyanide. Besides the usual multistep syntheses [3], an increasing number of organic chemical compounds are formed by multicomponent reactions (MCRs) that convert more than two educts directly into their products by one-pot reactions. In contrast, to the multistep syntheses, the MCRs need minimal work, and they have often quantitative yields. The chemistry of the MCRs officially began twelve years later, when Strecker [4] introduced the general formation of α-aminocyanides from ammonia, carbonyl compounds, and hydrogen cyanide.

Further, Ugi reaction was first reported by Ivar Ugi in 1959 [5], along with the Passerini reaction, it is classified as an isocyanide-based multicomponent reaction [6] usually, non-polar halogenated solvents prove detrimental, as most amines are insoluble, favoring the occurrence of the Passerini reaction [7]. The reaction is usually conducted in a polar protic solvent such as methanol, and some success in water has recently been shown [8] also mild reaction conditions allow inclusion of a variety of functionality as most interesting MCR are probably those including isocyanides [9,10] which embodied by the four components Ugi reaction. Rossen et al. have recently proposed the use of Ugi reaction as an alternative path for an intermediate along the synthesis of the Merck HIV protease inhibitor Crixivan (Indinavir) [11].

Since, the chemical nuclease activity of the various novel heterocycles were discovered in 1980s [12-14], Much literature survey show that, investigation on the interaction of DNA with small molecules are primary work in the design of new types of anticancer agents. Studying the interaction model and the mechanism of DNA cleavage, and exploring the application in antineoplastic medication, molecular biology and bioengineering were hotspots in recent year. When some kinds of drugs interacted with DNA, they could induce the breakage of DNA strands as a result the replication ability of cancer gene is destroyed. The interactions of peptide with DNA constitute a significant area of research which has attracted considerable attention from biochemists because studies have shown that they are involved biological systems and non-toxic.

Even though more number of work is being reported on DNA binding and cleavage by heterocyclic compounds, since they have some drawback due to their toxicity towards the normal cells. Therefore, still there is scope to develop new molecules which are biologically non-toxic towards the normal cells. With the above facts and in continuation of our quest we made effort to design and synthesis of quinoline applicated Ugi synthesis.

## MATERIALS AND METHOD

### Chemicals and instrumentations

The melting points were determined by capillary methods and are uncorrected. The IR spectra were recorded on a Nicollet model impact 400D FT-IR spectrometer (KBr pallets, 3 $cm^{-1}$ resolution), $^1H$ NMR spectra on a Bruker 400 MHz and mass spectra were recorded on ESMS Kratos pekompact SEQ V1.22 spectrometer and UV–visible absorption spectra were recorded using Shimadzu model. Amino acid methyl ester hydrochlorides were prepared by using methanol and thionyl chloride. CTDNA was purchased from Bangalore Gene, Bangalore, India. Tris–HCl buffer (5mMTris–HCl, 50MNaCl, pH = 7.2, Tris=Tris (hydroxymethyl) aminomethane) solution was prepared using deionized, double-distilled water. TLC analysis was carried out using the precoated silica gel $GF_{454}$ plates.

### General experimental procedure for the preparation of 2-Benzyl-5-(2 benzyloxycarbonylamino-3-Phenyl-propylamino)-4-(2-chloro-quinoline-3yl)-5-(2,2,2 trifluoro-acetoxy)-pentanoic acid methyl ester (6a-g).

The 2-chloro-3-formyl quinoline and amino acid methyl esters are mixed in a round bottomed flask, initially stir the reaction mixture for about 2 hr in presence of dry methanol, then add the freshly prepared N-protected amino acid isonitrile and Trifloro acetic acid and leave the reaction mixture to stir for about a day. Check the completion of the reaction by TLC and do the normal work up.

## RESULTS AND DISCUSSION

Recent trends in multicomponent reactions (MCRs) [17] constitute an ideal approach to transformations for generating complex molecular skeletons in one pot. While very impressive results have been registered in this area, we believe that an important tool of combinatorial chemistry, the multiple-component condensation (MCC), is currently being underexploited [18]. The Ugi four-component reaction (4-CR) [19], in which primary amines, oxo species, carboxylic acids, and isocyanides react is one of the most representative MCRs. Despite the importance of Ugi-type reactions in the synthesis of heterocyclic compounds, only scant attention has been paid to the reaction partners. Our systematic investigation of structure-function relationships using combinatorial chemical strategies may become good attractive research because several different research reports demonstrated the usefulness of Ugi four-component condensation reaction (U-4CR) in the synthesis of the PNA monomers [20]. Utilization of this above technique, made driving force for us to carryout Ugi application in quinolines to explore new era in quinoline chemistry.

In this work the **(6a-h)** was synthesized by U-4CR (**Scheme I**), from reaction of 2-chloro-3-formyl quinoline **(1),** Amino acid methyl ester (**2**), Trifloroacetic acid **(3)** and N- protected amino acid isocyanate (**4**) Methanol at room temperature afforded the four component adduct (**6a-h)** around 70% yield. The various results are summarized in (**Table 1).** In the four components of the reaction, isonitriles **(4)** has to be prepared just before use due to the instability. So an amino acid methyl ester was chosen in the Ugi reaction. This will provide a terminal for amino acid attachment in the final molecule. In this work, 2-chloro-3-formyl quinoline was synthesized by reported method using Villas Mayer hack reaction [21]. Finally, the **(6a-h)** was characterized by LC-MS, $^1$H NMR, and elemental analysis.

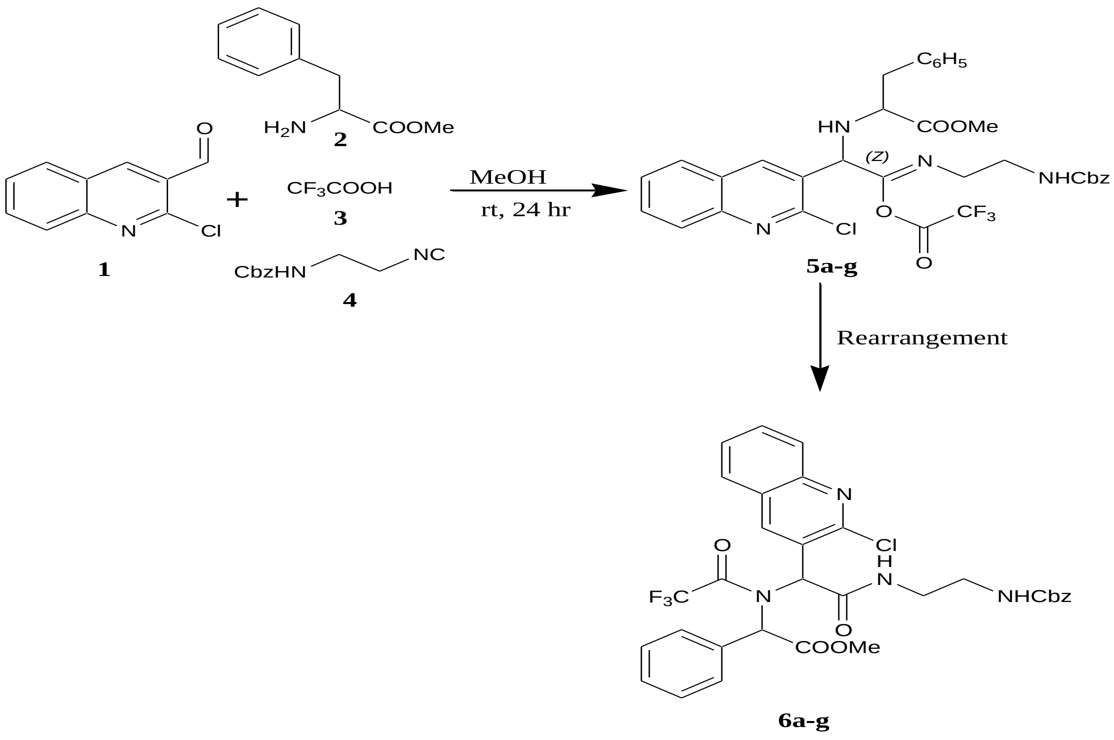
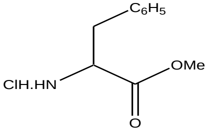
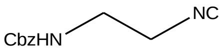
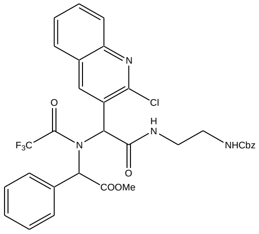
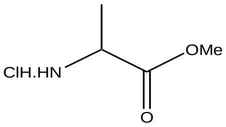
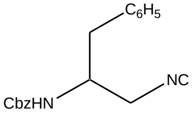
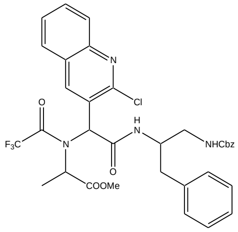
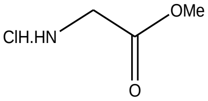
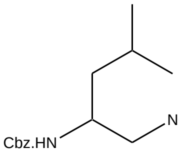
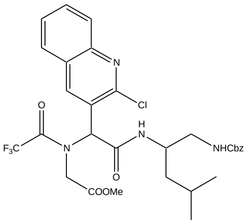
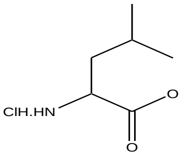
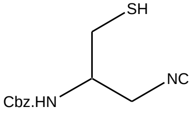
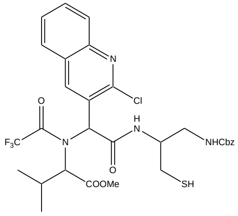
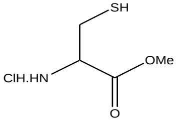
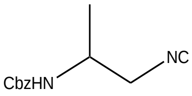
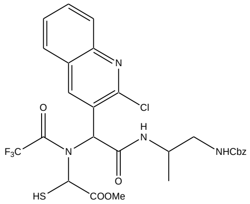
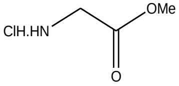
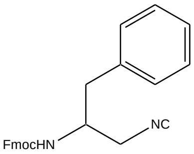
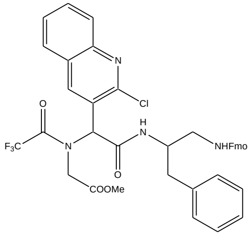
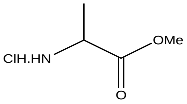
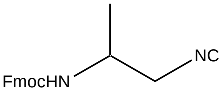
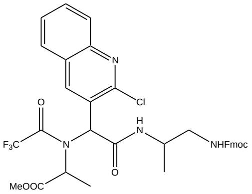


**Figure 1: Schematic representation of desired molecules (6a-g) through systematic pathway.**

## Table 1: List of desired molecules.

| Sl. No | Aldehyde | Ester | Isocyanate | Acid | Product |
|---|---|---|---|---|---|
| 6a | 1 |  |  | 3 |  |
| 6b | 1 |  |  | 3 |  |
| 6c | 1 |  |  | 3 |  |
| 6d | 1 |  |  | 3 |  |
| 6e | 1 |  |  | 3 |  |
| 6f | 1 |  |  | 3 |  |
| 6g | 1 |  |  | 3 |  |

**Compound 6a:** Light yellow solid, yield=70%, IR (ν) (KBr) 1648 (C=O), 3421 (NH), 1742 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 3.1 (d, 2H), 3.25 (d, 2H), 3.42 (m, 3H), 5.0 (d, 2H), 5.46 (s, 2H), 7.2-8.1 (m, 15H, Ar-H); m/z (%) [M] $^+$: [657] $^+$; Elemental analysis, found: C, 58.53; H, 4.31; N, 8.51. Calculated for $C_{32}H_{28}ClF_3N_4O_6$: C, 58.50; H, 4.30; N, 8.53.

**Compound 6b:** Light yellow solid, yield=68%, IR (ν) (KBr) 1653 (C=O), 3429 (NH), 1742 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.2 (m, 3H), 2.42 (d, 2H), 2.9 (d, 2H), 3.38 (m, 3H), 4.42 (s, 2H), 5.12 (d, 2H), 5.44 ( s, 1H), 7.2-8.0 (m, 15H, Ar-H); m/z (%) [M] $^+$: [685] $^+$; Elemental analysis, found: C, 59.60; H, 4.74; N, 8.20.Calculated for $C_{34}H_{32}ClF_3N_4O_6$: C, 59.61; H, 4.71; N, 8.18.

**Compound 6c:** Light yellow solid, yield=68%, IR (ν) (KBr) 1641 (C=O), 3414 (NH), 1736 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 0.9 (m, 6H), 1.22 (d, 2H), 1.64 (s, 1H), 2.9 (d, 2H), 3.36 (m,3H), 4.0 (m, 3H), 5.12 (d, 2H), 5.31 (s, 1H), 7.2-7.9 (m, 10H, Ar-H); m/z (%) [M] $^+$: [637] $^+$; Elemental analysis, found: C, 56.52; H, 5.08; N, 8.74. Calculated for $C_{30}H_{32}ClF_3N_4O_6$: C, 56.56; H, 5.06; N, 8.79.

**Compound 6d:** Light yellow solid, yield=64%, IR (ν) (KBr) 1649 (C=O), 3422 (NH), 1741 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 0.92 (m, 6H), 1.2 (s, 1H), 2.22 ( d, 2H), 2.48 (s, 1H), 3.0 (d, 2H), 3.36 (m, 3H), 4.31 (s, 2H), 5.14 (d, 2H), 5.4 (s, 1H), 7.1-7.8 (m, 10H, Ar-H); m/z (%) [M] $^+$: [669] $^+$; Elemental analysis, found: C, 53.87; H, 4.80; N, 8.58. Calculated for $C_{30}H_{32}ClF_3N_4O_6S$: C, 53.85; H, 4.82; N, 8.57.

**Compound 6e:** Light yellow solid, yield=67%, IR (ν) (KBr) 1654 (C=O), 3430 (NH), 1750 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.1 (d, 3H), 1.29 (s, 1H), 3.16 (d, 2H), 3.35 (m, 3H), 4.1 (s, 1H), 5.34 (m, 3H), 5.61 ( s, 1H), 7.2-7.9 (m, 10H, Ar-H); m/z (%) [M] $^+$: [627] $^+$; Elemental analysis, found: C, 51.75; H, 4.15; N, 8.96. Calculated for $C_{27}H_{26}ClF_3N_4O_6S$: C, 51.72; H, 4.18; N, 8.94.

**Compound 6f:** Light yellow solid, yield=69%, IR (ν) (KBr) 1646 (C=O), 3412 (NH), 1729 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 2.41 (d, 2H), 3.25 (d, 2H), 3.42 (m, 3H), 3.92 (d, 2H), 4.5 (m, 4H), 5.34 (s, 1H), 7.3-8.1 (m, 18H, Ar-H); m/z (%) [M] $^+$: [835] $^+$; Elemental analysis, found: C, 66.17; H, 4.54; N, 6.75. Calculated for $C_{46}H_{38}ClF_3N_4O_6$: C, 66.15; H, 4.59; N, 6.71.

**Compound 6g:** Light yellow solid, yield=71%, IR (ν) (KBr) 1649 (C=O), 3429 (NH), 1741 (COOMe) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.3 (m, 6H), 3.22 (d, 2H), 3.42 (m, 3H), 4.5 (m, 5H), 5.46 (s, 1H), 7.2-7.9 (m, 13H, Ar-H); m/z (%) [M] $^+$: [697] $^+$; Elemental analysis, found: C, 60.28; H, 4.60; N, 8.01.Calculated for $C_{35}H_{32}ClF_3N_4O_6$: C, 60.30; H, 4.63; N, 8.04.

## DNA INTERACTION EXPERIMENT

### *Spectral measurements*

UV–visible absorption spectra were determined in a SHIMADZU, UV-1650 PC recording spectrophotometer using quartz cuvettes of 10mm light-path. The parameter of interaction between quinoline thioureidopeptidyl ester with CT-DNA was determined spectrophotometrically using a Beckman 25 double-beam spectrophotometer. Aliquots of a concentrated DNA solution (0.18–1.125 mM) were added to a cuvette filled with (**6a**) solution (12–25µM) and thoroughly mixed. Extreme care was taken to ensure that optical reference solutions were prepared in an identical manner. The binding data were expressed in the form of a Scatchard plot [15]. The variables r (moles of ligand bound/mole of nucleotides) and C (the molar concentration of free drug) were calculated from the absorption measurements according to the method of Peacocke and Skerrett [16]. The intrinsic binding constant $K_b$ and the maximum number of available binding sites/nucleotide (n) were deduced from Scatchard plot.

Absorption:

$$(DNA) / (\varepsilon_a - \varepsilon_f) = (DNA) / (\varepsilon_b - \varepsilon_f) + 1 / (\varepsilon_a - \varepsilon_f) \text{ ---------------------- } (1)$$

Where $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ are the apparent, free, and bound extinction coefficients at 319nm for Trifluoro-Quinoline-Esters respectively. A plot of $[DNA]/(\varepsilon_b - \varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a γ intercept equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, where $K_b$ is the ratio of the slope to the γ intercept.

## DNA PHOTOCLEAVAGE EXPERIMENTS

The experiments were performed in a volume of 25 µL containing pUC19 DNA in 5 µmol/L phosphate buffer contained 10 µmol/L NaCl, pH 7.4, in the presence of different concentrations (100-600 µmol/L) of molecule. Immediately prior to irradiating the samples with UV light, $H_2O_2$ was added to a final concentration of 2.5 µmol/L.

The reaction volumes were held in caps of polyethylene microcentrifuge tubes, which were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 0.5 ml of a mixture containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer (45 µmol/L Tris-borate, 1 µmol/L EDTA). Untreated pUC19 DNA was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/ml) and photographed under UV light.

## DNA Binding Studies

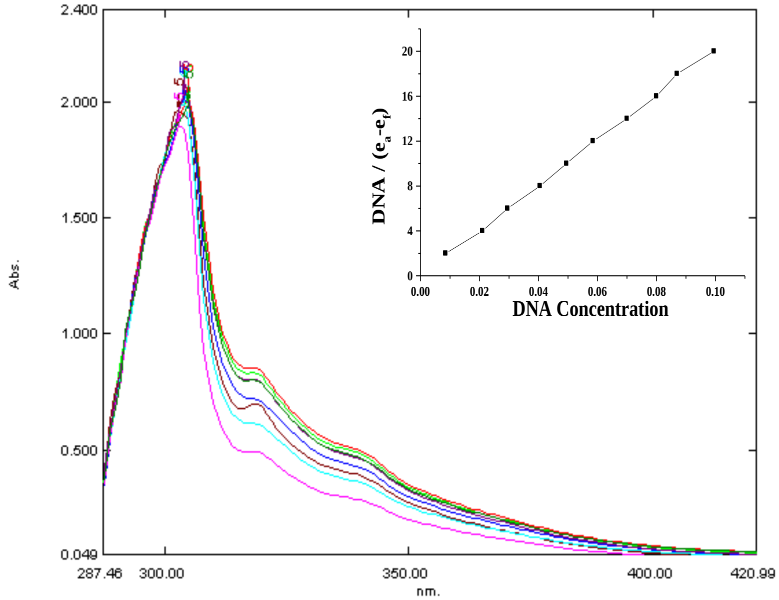


**Figure 2: Absorption spectra of trifluoro-quinoline-ester in Tris–HCl buffer upon addition of DNA (6a): = 0.5µM, [DNA] = 0–100µM. Showing variation in absorption with increase in concentration of [DNA]. Inner plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for titration of DNA.**

The DNA binding studies were characterized by absorbance maxima at 298nm for trifluoro-quinoline-esters (**6a**). The interaction of (**6a**) with DNA resulted in the decrease of absorption intensity accompanied by a shift towards higher wavelengths from 298nm. The addition of increasing higher concentration of DNA led to hypochromic and bathochromic (red shift) changes in its visible absorption spectra (**Figures I**). Around 12–14% reduction (bathochromism) of absorption was observed at 298nm peak maximum in the presence of an excess of calf thymus DNA. The lowest observation value observed in spectral changes (including red shift and hypochromicity) were used to evaluate intrinsic binding constant ($K_b$), it observed $4.2\times10^{-4}$ $M^{-1}$ which are consistent with the intercalation of (**6a**) with DNA base pairs[20].

## DNA Cleavage Studies

Nuclease activity exhibited by certain long chain of fused planar heterocycles in the presence of hydrogen peroxide or ascorbic acid is attributed to the participation of hydroxyl radical in DNA cleavage[21]. The naturally occurring supercoiled circular form of pUC19 DNA (Form I), when nacked, gives rise to an open circular form (Form II), relatively fast migration is observed for form I and slow migration for form II. These qualitative findings could be quantified by densitometric analysis of the bands originating from SC and NC plasmids. Bands from the linear form, although clearly visible on the gel, were difficult to quantify. Large errors arise on weaker bands because the definition of the background is somewhat arbitrary in those cases. Therefore, parameters for quantification were chosen such that only the SC and NC bands were included in the procedure. The sum of intensity of both bands was standardized to 100% in all lanes

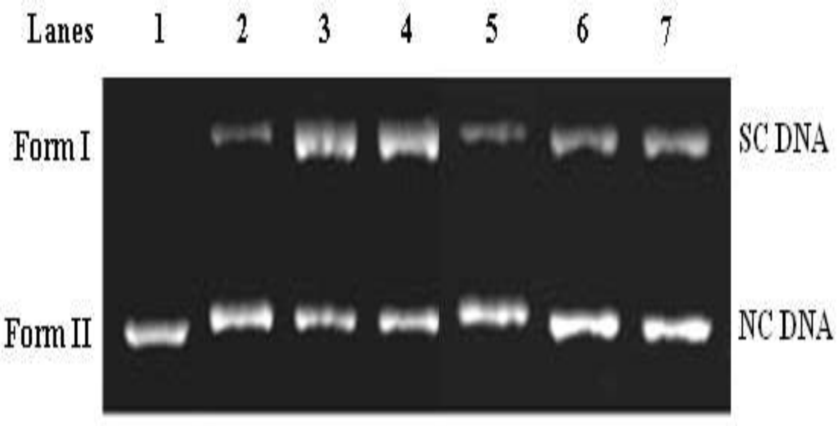


**Figure 3: Effects of 6a at various concentrations (20-120 µmol) on the pUC 19 supercoiled DNA against $^{\cdot}$OH generated by photolysis in presence of $H_2O_2$/ascorbic acid. Lane 1, untreated DNA (control); lane 2, DNA + $H_2O_2$ (20µM); lane 3, DNA + 6a (40 µM); lane 4, DNA + 6a (60 µM); lane 5, DNA + 6a (80 µM); lane 6, DNA + 6a (100 µM); lane 7, DNA + 6a (120 µM).**

Figure II shows the gel electrophoretic separation of plasmid pUC19 DNA treated with compound **6a**. Control experiments suggest that untreated DNA and DNA incubated with compound **6a** show significant DNA cleavage (lanes 2… 7). However, in the presence of peroxide, the compound **6a** convert the supercoiled plasmid DNA to a mixture of supercoiled (Form I) and nicked (Form II) DNA. The generation of hydroxyl radical mediated by the compound **6a** results in DNA cleavage activity. From the **(Figure II)** it is clear that in the presence of peroxide, the compound **6a** exhibit nuclease activity due to hydroxyl radical participation in the DNA cleavage activity.

## CONCLUSIONS

In summary, we present best and efficient method in Ugi four-component reaction for the synthesis of Trifluoro-Quinoline-Esters derivatives in one pot. Described protocol offers scope for further work involving a variety of substrates with varied substituents. Further, the obtained results of DNA binding studies shows good interaction of trifloro quinoline esters towards CT/pUC 19 DNA.

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