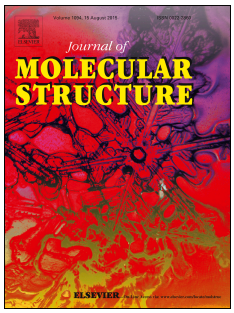

Synthesis, characterization, crystal structure and anticancer activity of tetrahydro-quinolines using silica iodide as a heterogeneous catalyst

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Authors Contribution

Dr. Ramesh K B

Conceptualization, Methodology, Validation, Writing and Formal analysis

Omkarmurthy B M

Resources, Software and Crystal structure interpretation

Dr. Srinivas M

Visualization, Verification, Data Curation, Supervision and Investigation

Synthesis, characterization, crystal structure and anticancer activity of Tetrahydro-quinolines using Silica Iodide as a heterogeneous catalyst

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Abstract

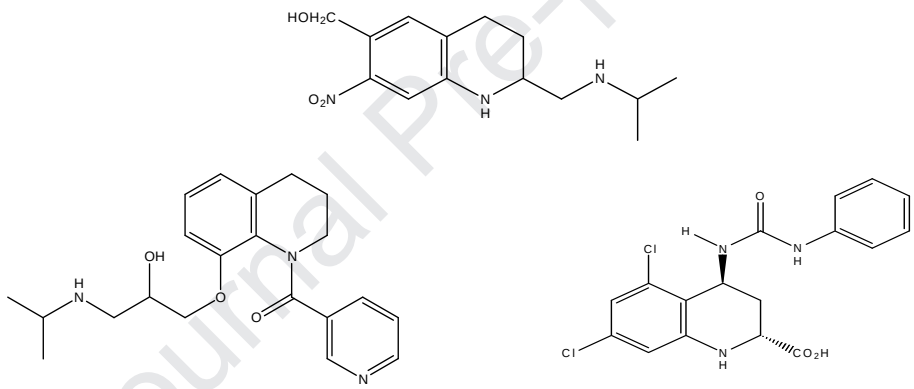
An efficient synthesis of tetrahydroquinolines using silica iodide as heterogeneous catalyst. Ethyl acetoacetate, dimedone, ammonium acetate with suitable aromatic aldehydes in ethanol was reported. Silica iodide (SiO_2-I) acts as an efficient heterogeneous catalyst to afford the products in excellent yield in short reaction duration and reusable catalyst. The synthesized 4d and 4h compounds were characterized by the single crystal X-ray diffraction method. Further, the prepared quinoline derivatives showed potent anti-cancer activity against HepG2 and MCF-7 cell lines. Docking study was carried out to evaluate the binding affinity of the synthesized compounds and the standard drug doxorubicin with Estrogen Receptor (ER).

Keywords: Synthesis: Tetrahydroquinolines, Silica iodide, crystal structure, anti-cancer activity, docking study.

1. INTRODUCTION

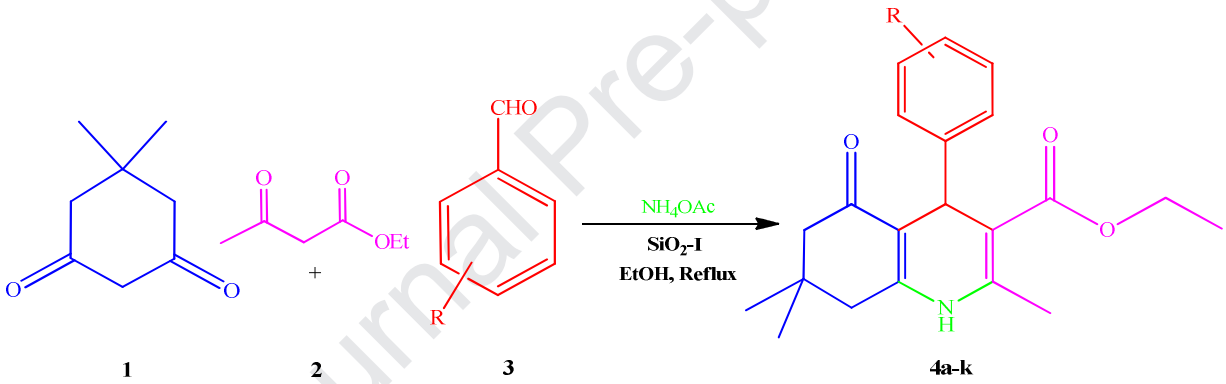
In recent years, the advanced research on development of “one-pot reactions” represents a great

challenge in organic synthesis predominantly in the development of modern drugs [1]. Quinoline is a heterocyclic structural unit which is found to occur in many natural alkaloids, therapeutics and in other synthetic analogues [1]. Quinolines show a wide spectrum of biological activities such as antiproliferative, antiplasmodial, antimalarial, antibacterial, and anticancer activities due to the variety of pharmacological properties [3]. Quinolines represent a large family of heterocyclic compounds which find application in the design of various medicinally important compounds and hence, much attention has been focused on the synthesis of a variety of quinoline derivatives **[**4]. There are few methods reported on the synthesis of the polyhydroquinolines (a, b and c) in the literature [5]. The reported methods have significant drawbacks such as use of relatively expensive and

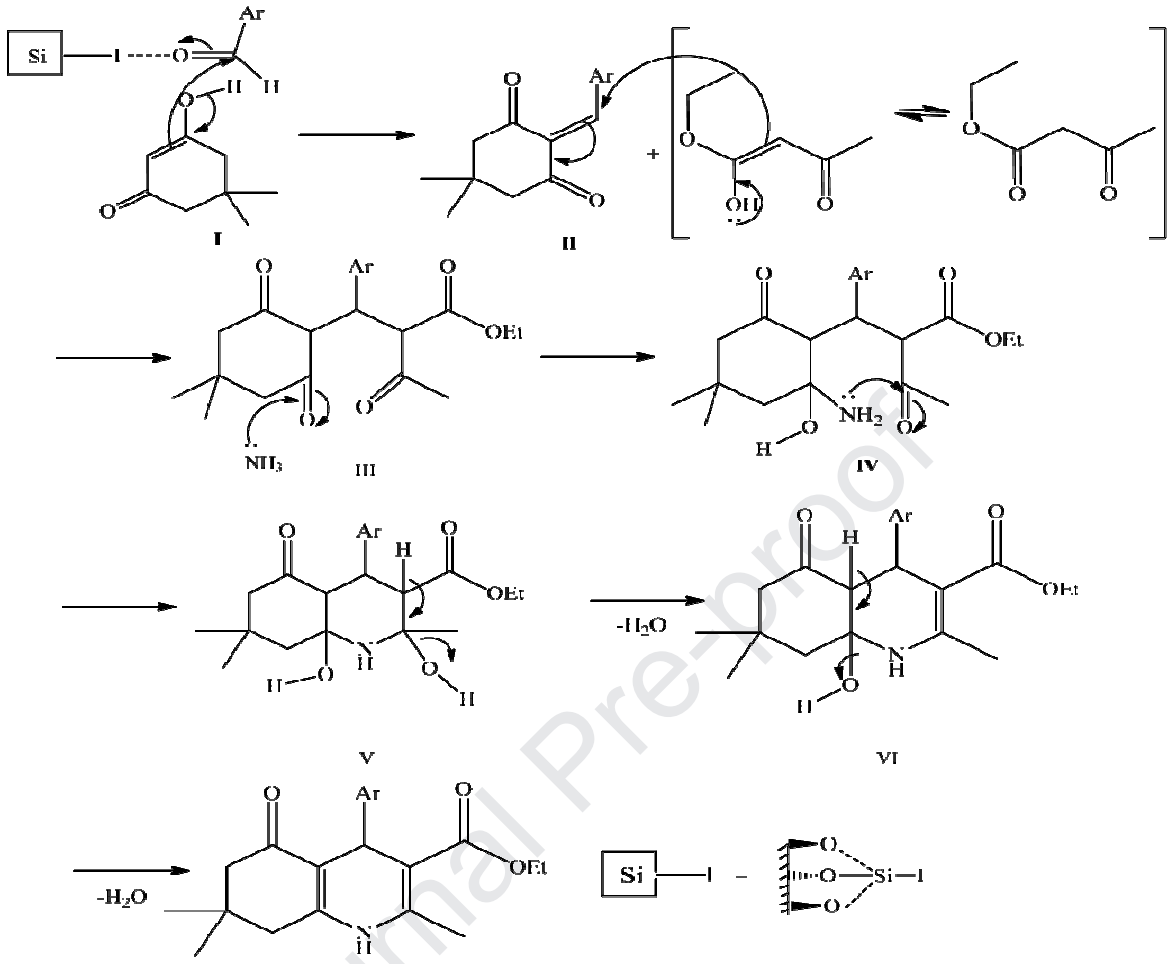


hazardous reagents, low yields and involve drastic reaction conditions. Preparation of quinoline derivatives by a one-pot multi-component strategies is a challenging and is in great demand. Hence, moreover there is scope for investigations on such reactions in order to improve the yields by use of mild and less harsh reaction conditions. Recently, heterogeneous catalysis has gained much attention in the various fields of science including the synthetic organic chemistry compared to homogeneous catalysis. Heterogeneous catalysts have many advantages since it ex-

hibits the high atom efficiency, recovery and recyclability. This research article deals with synthesis of one-pot multi-component quinoline derivatives by the use of SiO_2-I as a novel heterogeneous catalyst [6-8]. Further, an attempt is made to study the Crystal structure of the synthesized 4d and 4h compounds were characterized by the single crystal X-ray diffraction method. Applications for anticancer activity and molecular docking of synthesized compounds and their behavior. To the best of our knowledge here we report SiO_2-I heterogeneous catalyst is simple, environment friendly and efficient method for the one-pot four-component synthesis of tetrahydroquinoline derivatives were presented. Crystal structure of 4d and 4h and analyzed and correlated with anticancer activity and docking. Synthesized compounds are evaluated with reference to the standard drug doxorubicin (Scheme 1).



Scheme 1: SiO_2- I catalyzed synthesis of Tetrahydroquinolines



The Plausible mechanisms for the synthesis of Tetrahydroquinolines

2. EXPERIMENTAL

2.1. Materials and methods

All the chemicals were commercially available and used without further purification, except liquid aldehydes which were distilled before use. All yields refer to yield of the isolated products after purification. All the products were characterized by the IR, ^{1}HNMR, ^{13}CNMR, Mass spectral and CHN analyses. Melting points were determined on a RAAGA make melting point appa-

ratus. Nuclear magnetic resonance spectra were obtained from Bruker AMX instruments in $CDCl_3$ using TMS as an internal standard [400 MHz and 100 MHz for ^{1}HNMR for ^{13}CNMR respectively]. ESI-MS analysis was carried out using ESI-Q TOF instrument. The crystal data were collected at Xcalibur, Eos, Nova diffractometer. The crystal was kept at 293(2) K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using CGLS minimization [9-10]. For biological assays, the HepG2 and MCF-7 cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune, INDIA.

3. RESULTS AND DISCUSSION

Primarily, one pot four-component reaction of 4-methoxybenzaldehyde (1 mmol), dimedone (1 mmol), ethyl acetoacetate (1 mmol), ammonium acetate (1.5 mmol) in EtOH (5 mL) was carried out with different catalysts are Amberlite IR-120H, $CeCl_3$, $SnCl_2$, Na_2CO_3 and A4 size H^+ molecular sieves at reflux temperature of the solvent and were found that, SiO_2-I is best in terms of yield and duration of the reaction (**Entry 9 Table 1**).

Entry	Catalyst	Time(h)	Yield[d] (%)
1	Na_2CO_3[b]	6	30
2	$Ba(OH)_2$[b]	6	60
3	$SnCl_2$[b]	8	50
4	Acidic Molecular sieves A4[c]	9	40
5	Amberlite IR120[c]	13	20
6	$CeCl_3$[b]	12	30
7	SiO_2[c]	4	60
8	SiO_2-Cl[c]	3.5	70

9	SiO_2-I[c]	2.5	90

Table 1. Effect of various catalysts on the synthesis of 4c[a]

[a]**Reaction condition:** 4-methoxybenzaldehyde (1 mmol), dimedone (1 mmol), ethyl acetoacetate (1 mmol), ammonium acetate (1.5 mmol) and EtOH (mL); [b]10 mol%; [c]0.1g; [d]isolated yield.

3.1 Chemistry

We started the research work by examining the reaction of 4-methoxybenzaldehyde with dimedone, ethyl acetoacetate and ammonium acetate to get 4-(4'-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (**4c**) in EtOH in the presence of catalytic SiO_2-I as a heterogeneous catalyst refluxed around 2–3h and yields 90 %. To understand the importance of this method, performed further all the reactions using SiO_2-I (0.1g) in EtOH at reflux, and it was found that, SiO_2-I can efficiently catalyze the reaction between dimedone, ethyl acetoacetate, ammonium acetate, and different aromatic aldehydes to give excellent yield of the desired products within 2-3 h respectively. The data presented in the Table 2, it is clear that, the method is effective for both electron withdrawing and electron donating aromatic aldehydes [11-12]. Then, we carried out the reaction using aliphatic aldehydes (**Table 2**, entries 10 and 11), and it was found that, there was no product formation even after 15 h.

Entry	Aldehydes	Product	Time (h)	Yield[a] (%)	Mp (°C)	
					Found	Reported
1	2,3,4-$MeOC_6H_2CHO$	**4a**	2.5	90	180−182[†]	–
2	3-Br,4-$MeOC_6H_3CHO$	**4b**	2.5	87	250–251[†]	–

3	4-$MeOC_6H_4CHO$	**4c**	2.5	90	145–147	145−147 [9]
4	2-IC_6H_4CHO	**4d**	3.0	86	181–183†	–
5	3-HO,4-$MeOC_6H_3CHO$	**4e**	2.3	85	199–201	199–201[10]
6	3,5-BrC_6H_3CHO	**4f**	2.4	82	255–257†	–
7	3,4- ClC_6H_3CHO	**4g**	2.8	80	216–218	216−218[10]
8	3-F,4-ClC_6H_3CHO	**4h**	2.6	89	220–222†	–
9	4-HOC_6H_4CHO	**4i**	3.0	87	238–240	238−240[10]
10	HCHO	**4j**	15.0	ND	–	–
11	CH_3CHO	**4k**	15.0	ND	–	–

[a]Isolated yield; †Novel compounds

Table 2. Synthesis of the Tetrahydroquinolines (4a–k)

3.2 Typical experimental procedure for the synthesis of Tetrahydroquinolines:

A mixture of aldehyde (1 mmol), dimedone (1 mmol), ethyl acetoacetate (1 mmol) and ammonium acetate (1.5 mmol) was taken in EtOH (5 mL) mixed well and then SiO_2-I (1.7 mmol) was refluxed for 2-3h at 80°C. The course of the reaction was monitored by TLC [EtOAc: Hexane (3:7)]. After the completion of the reaction mixture was cooled and poured onto crushed ice. The obtained solid product was filtered along with the catalyst the residue was dissolved in ethanol and filtered to separate the catalyst. Catalyst was kept aside for further use. The crude product obtained after the removal of the solvent under vacuum. Further the crude product was crystallized from ethanol to get the pure products. The spectral and analytical data for some of the prepared compounds is presented in the **Table 2.**

3.3 Spectral and analytical data of selected synthesized compounds

3.3.1 **2,7,7-Trimethyl-5-oxo-4-(2',3',4'trimethoxyphenyl)-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4a):**

Colorless solid, Mp: 180–182 °C; IR (KBr): ν 3350 (N-H), 1712 (C=O); 1700, 1642, 1611, 1499, 1422, 1305, 1214, 1053, 1011 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (s, 3H, CH_3,C(11) H), 1.06 (s, 3H, CH_3,C(12)H), 1.95 (t, J = 6.8Hz, 3H,C(21) H), 2.21(m, 4H, $2CH_2$, C(6) C(8) H), 2.35 (s, 3H,CH_3, C(1) H), 3.76–3.89 (s, 9H, 3 × OCH_3, C(22)C(23)C(24) H), 4.04–4.06 (q, J = 2.4 Hz, 2H,C(20) H), 5.11 (s, 1H, CH,C(4) H), 6.52 (d, J = 8.8 Hz, 1H,C(14) H), 6.97 (d, J = 6.8 Hz, 1H,C(15) H), 10.24 (s, 1H, N-H) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 14.30 (C-21), 19.42(C-1), 27.03(C-11, C-12), 29.54 (C-4), 32.62 (C-7), 50.95 (C-8), 55.84 (C-6,C-24), 59.73 (C-22), 60.41 (C-24), 60.50 (C-23),100.10 (C-10) 105.50 (C-3), 106.28 (C-15), 111.12 (C-13), 125.70 (C-14), 132.46 (C-17), 142.10 (C-2), 142.94 (C-9),149.44 (C-16), 152.11 (C-18), 168.10 (C-19,C=O), 195.72 (C-5, C=O) ppm; ESI-MS:[M] 429.5; Anal. Calcd. $C_{24}H_{31}NO_6$ (%): C, 67.11; H, 7.27; N, 3.26; Found C, 66.01; H, 7.07; N, 3.21.

3.3.2 4-(3'-Bromo-4'-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylicacid ethyl ester (4b):

Colorless solid, Mp: 250–251 °C; IR (KBr): ν 3346 (N-H), 1716 (C=O); 1708, 1609, 1521, 1489, 1410, 1300, 1207, 1063, 1020 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H, CH_3, C(11) H), 1.07 (s, 3H, CH_3, C(12) H), 1.21 (t, J = 7.2 Hz, 3H, C(21) H), 2.36–2.44 (m, 4H, $2CH_2$, C(6) C(8) H), 2.70 (s, 3H, CH_3, C(1) H), 3.80 (s, 3H, OCH_3, C(22) H), 4.05–4.10 (q, J = 7.2 Hz, 2H, C(20) H), 4.96 (s, 1H, CH, C(4) H), 6.66 (d, J = 8.0 Hz, 3H, C(14) H), 6.78 (d, J = 8.0 Hz, 1H, C(15) H), 10.28 (s, 1H, NH) ppm; ^{13}CNMR (100MHz,$CDCl_3$): δ 14.25 (C-21) , 17.09 (C-7), 18.90 (C-1), 26.80 (C-11,C-12), 29.83 (C-4), 48.15 (C-8),54.17 (C-22), 58.07 (C-6), 59.37 (C-20), 85.82 (C-10), 108.08 (C-17), 122.67 (C-3), 123.52 (C-15), 128.08 (C-14), 131.43 (C-13), 137.19 (C-18), 145.35 (C-2), 148.98 (C-9), 156.92 (C-16), 164.25 (C-19,C=O),197.64 (C-

5,C=O) ppm. ESI-MS:[M+1]448.1; Anal.Calcd for $C_{22}H_{26}BrNO_4$: C, 58.94; H, 5.85; N, 3.12. Found: C, 57.33; H, 5.05; N, 3.10.

3.3.3 4-(2'-Iodophenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4d):

Colorless solid, Mp: 181–183 °C; IR (KBr): ν 3339 (N-H), 1723 (C=O); 1709, 1600, 1545, 1408, 1400, 1299, 1207, 1083, 1030 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.93 (s, 3H, CH_3, C(11) H), 1.06 (s, 3H, CH_3 C(12) H), 1.18 (t, J = 6.4 Hz, 3H, C(21) H), 2.18–2.22 (m, 4H, $2CH_2$, C(6)C(8) H), 2.38 (s, 3H, CH_3, C(1) H), 4.03−4.08 (q, J = 6.8 Hz, 2H, C(20) H), 4.97 (s, 1H, CH, C(4) H), 6.71 (d, J = 8.0 Hz, 2H, Ar-H, C(18) H), 7.18 (d, J = 8.0 Hz, 2H, Ar-H, C(15) H), 9.51 (s, 1H, NH) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 14.71 (C-21), 19.71 (C-1), 27.57(C-11,C-12), 29.96(C-4), 50.99(C-6,C-8),60.26 (C-20), 98.74 (C-14), 102.20 (C-3),106.92 (C-10),112.1(C-17),113.6(C-16), 129.4(C-18), 140.0(C-2,C-15), 143.9(C-9), 150.4(C-13), 168.0 (C-19, C=O), 196.3(C-5, C=O) ppm; ESI-MS: [M+1] 466.08; Anal.Calcd for $C_{21}H_{24}$ INO_3 : C, 54.20; H, 5.20; N, 3.01. Found: C, 53.22; H, 5.12; N, 2.73.

3.3.4 4-(3'-Hydroxy-4'-methoxy-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4e):

Colorless solid, Mp: 199–201°C; IR (KBr): ν 3310 (N-H), 1746 (C=O); 1699, 1602, 1525, 1412, 1401, 1229, 1203, 1023, 998 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H, CH_3, C(11) H), 1.07 (s, 3H, CH_3, C(12) H), 1.21 (t, J = 7.2 Hz, 3H, C(21) H), 2.31 (m, 4H, $2CH_2$, C(6) C(8) H), 2.40 (s, 3H, CH_3, C(1) H), 2.62 (s, 1H, C(15) OH), 3.80 (s, 3H, OCH_3, C(22) H), 4.05−4.10 (q, J = 7.2 Hz, 2H, C(20) H), 4.95 (s, 1H, CH, C(4) H), 6.66–6.80 (m, 3H, Ar-H, C(14,17,18) H), 8.50 (s, 1H, NH) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 13.90 (C-21), 17.29 (C-7), 18.20 (C-1), 27.81 (C-11, C-12), 29.72 (C-4), 47.51 (C-8), 51.19 (C-6), 55.54 (C-22), 59.64 (C-20), 100.23 (C-3),

108.23 (C-10), 113.90, 114.08 (C-17), 116.23 (C-14), 128.23 (C-18), 130.26 (C-13), 136.91 (C-9), 142.50 (C-2,C-15), 145.02 (C-16), 160.26 (C-19,C=O), 196.94 (C-5, C=O) ppm; ESI-MS: [M+1] 386.19; Anal.Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 67.91; H, 6.78; N, 3.61.

3.3.5 4-(3',5'-Dibromo-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4f):

Colorless solid, Mp: 255–257 °C; IR (KBr): ν 3300 (N-H), 1796 (C=O); 1599, 1501, 1435, 1400, 1399, 1209, 1200, 1013, 910 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.96 (s, 6H, 2 CH_3 ,C(11)(12) H), 1.08 (t, J = 7.6 Hz, 3H, CH_3, C(21) H), 2.23 (m, 4H, $2CH_2$, C(6)C(8) H), 2.33 (s, 3H, CH_3, C(1) H), 4.06−4.08 (q, J = 4.0 Hz, 2H, C(20) H), 4.96 (s, 1H, CH, C(4) H), 6.72–6.75 (d, J = 12.0 Hz, 2H, Ar-H, C(16)C(14) H), 7.39 (s, 1H, Ar-H, C(18) H) 9.69 (s, 1H, NH) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 13.30 (C-21), 17.70 (C-7), 18.90 (C-1), 26.80 (C-11,C-12), 30.91 (C-4), 55.82 (C-6), 59.95 (C-20), 100.10 (C-3), 108.02 (C-10), 122.47 (C-15), 128.45 (C-18), 128.52 (C-17), 129.75 (C-16), 130.14 (C-14), 137.51 (C-13), 145.66 (C-9), 153.06 (C-2), 160.15 (C-19, C=O), 194.24 (C-5, C=O) ppm; ESI-MS: [M] 495.00; Anal.Calcd for $C_{21}H_{23}Br_2NO_3$: C, 50.73; H, 4.66; N, 2.82. Found: C, 50.32; H, 4.02; N, 2.32.

3.3.6 4-(3',4'-Dichloro-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4g):

Colorless solid, Mp: 216–218 °C; IR (KBr): ν 3386 (N-H), 1711 (C=O), 1704, 1662, 1615, 1509, 1438, 1365, 1234, 1153, 1044 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.91 (s, 6H, $2CH_3$ C(11) C(12) H), 1.02 (t, J = 7.6 Hz, 3H, CH_3, C(21) H), 2.16 (m, 4H, $2CH_2$, C(6)C(8) H), 2.25 (s, 3H, CH_3, C(1) H), 3.99−4.01 (q, J = 6.8Hz, 2H, C(20) H), 5.05 (s, 1H, CH, C(4) H), 6.94−7.26 (m, 3H, Ar-H, C(14)C(17)C(18) H), 10.49 (s,1H,NH) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 14.25 (C-

21), 16.08 (C-7), 19.48 (C-1), 27.65 (C-11, C-12), 29.28 (C-4), 50.90 (C-8), 54.20 (C-6), 59.76 (C-20), 102.61 (C-3), 109.08 (C-10), 127.29 (C-18), 130.10 (C-17), 132.16 (C-16, C-14), 138.11 (C-13, C-15) ppm, 139.32 (C-2), 145.10 (C-9), 167.55 (C-19, C=O), 195.64 (C-5, C=O) ppm. ESI-MS: [M] 407.1; Anal.Calcd for $C_{21}H_{23}Cl_2NO_3$: C, 61.77; H, 5.68; N, 3.43. Found: C, 60.32; H, 5.02; N, 3.32.

3.3.7 4-(4'-Chloro-3'-fluoro-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylicacid ethyl ester (4h):

Colorless solid, Mp: 220–222 °C; IR (KBr): ν 3429 (N-H), 1709 (C=O), 1649, 1644, 1408, 1336, 1287, 1129, 1010,810 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.93 (s, 3H, CH_3, C(11) H), 1.08 (s, 3H, CH_3, C(12) H), 1.17 (t, J = 7.2 Hz, 3H, C(21) H), 2.20–2.23 (m, 4H, $2CH_2$, C(6)C(8) H), 2.40 (s, 3H, CH_3, C(1) H), 3.31 (s, 1H, NH), 4.05–4.07 (q, J = 7.2 Hz, 2H, C(20) H), 5.02 (s, 1H, CH, C(4) H), 7.04–7.07 (m, 2H, Ar-H C(14) H), 7.21−7.23 (m, 2H, Ar-H, C(17) C(18) H) ppm; ^{13}CNMR (100MHz, $CDCl_3$): δ 14.32 (C-21),17.04 (C-7), 19.48 (C-1), 27.24 (C-11, C-12), 29.48 (C-4), 50.76 (C-8), 54.71 (C-6), 60.10 (C-20), 105.28 (C-3), 111.49 (C-10), 116.32 (C-16), 118.18 (C-14), 124.70 (C-18), 129.85 (C-17), 144.27 (C-2), 148.25 (C-13), 148.69 (C-9), 156.71 (C-15), 167.18 (C-19, C=O), 195.62 (C-5, C=O) ppm; ESI-MS: [M+1] 391.2; Anal.Calcd for $C_{21}H_{23}ClFNO_3$: C, 64.37;H,5.92; N, 3.57. Found:C,63.21;H,5.31;N,3.20.

4. Single crystal X-ray diffraction

The crystal structures of compounds 4d and 4h was determined by single crystal X-ray diffraction. The crystal data of 4d and 4h structure refinement parameters and selected bond parameters (Table 3). Refinement carried was full matrix least-squares on F^2. The hydrogen atoms were placed at calculated positions in the riding model approximation, their temperature factors were set to 1.2 times those of the equivalent isotropic temperature factors of the present atoms. All

other non-hydrogen atoms were refined anisotropically. The molecular structures, packing molecules and orientation of the planes containing the ring structures for 4d compound (Figure A, B and C) and 4h compound (Figure D, E and F). The SXRD study of 4d has revealed that tetrahydroquinoline-3-carboxylic acid ethyl ester is connected with iodide to form. The Molecular formula is C_{43} H_{51} I_2 N_2 O_6; Analysis made at 293.71 K: triclinic, space group P-1 with Z = 2, T = 293(2) K, μ(MoKα) = 1.519 mm^{-1} and Dcalc = 1.473 g/ cm^{-3}. The structure was solved by direct methods and refined to a standard discrepancy index of R = 0.0688 and Rw = 0.1433 for 2437 reflections with F 2σ (F) and a goodness of fit on F^2 = 1.010 (Table 4). Anisotropic displacement parameters can be visualized with a displacement ellipsoid plot (ORTEP) drawn at 30% probability level, indicates that the chance of finding the atomic nucleus within the plotted ellipsoid is 30%.

The SXRD study of 4h as revealed that tetrahydroquinoline-3-carboxylic acid ethyl ester is connected with fluorine and chlorine to form. The molecular formula is $C_{21}H_{23}ClFNO_3$; Analysis made at 293.71 K: orthorhombic, space group Pbcn with Z = 8, T = 293(2) K, μ (MoKα) = 0.217mm^{-1} and Dcalc = 1.281 g/ cm^{-3}. The structure was solved by direct methods and refined to a standard discrepancy index of R = 0.1133 and Rw = 0.2585 for 3716 reflections with F 2σ (F) and a goodness of fit on F^2 = 1.093 (Table 5). Hydrogen bonding interaction of C-H-N, N-H-O and N-H-N (4d and 4h) are 0.97, 0.85 and 0.86 respectively (Table 4 and Table 5).

Empirical formula	**C_{43} H_{51} I_2 N_2 O_6**	**C_{21} H_{23} Cl F N O_3**
Formula weight	945.65	391.85
Temperature/K	293(2)	293(2)

Crystal system	Triclinic	Orthorhombic
Space group	P-1	Pbcn
a/Å	13.281(2)	18.414(2)
b/Å	13.524(2)	15.599(2)
c/Å	14.772(2)	14.143(2)
α/°	90	90
β/°	65	90
γ/°	64	90
Volume/Å^3	2135.0(6)	4062.4(9)
Z	2	8
ρ_{calc}g/cm^3	1.471	1.281
μ/mm^{-1}	1.519	0.217
F(000)	954	1648
Crystal size/mm^3	0.480 x 0.060 x 0.020	0.260 x 0.140 x 0.140
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2θ range for data collection/°	2.835 to 25.348°.	2.881 to 25.350°.
Index ranges	-15<=h<=15, -16<=k<=16, -17<=l<=15	-16<=h<=22, -18<=k<=16, -12<=l<=17
Reflections collected	14092	15119
Independent reflections	7785 [R(int) = 0.0421]	1356 [R_{int} = 0.0188, R_{sigma} = 0.0333]
Data/restraints/parameters	7785 / 71 / 510	3716 / 31 / 245
Goodness-of-fit on F^2	1.017	1.010
Final R indexes [I>=2σ (I)]	R1 = 0.0656, wR2 = 0.1146	R_1 = 0.0559, wR_2 = 0.1691
Final R indexes [all data]	R1 = 0.1464, wR2 = 0.1369	R1 = 0.2585, wR2 = 0.3607
Largest diff. peak/hole / e Å^{-3}	0.861 and -0.394	0.474 and -0.382
CCDC	1993485	1993402

Table 3. Crystal data and structure refinement for 4d and 4h

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...O(1)#1	0.86	1.99	2.845(6)	174.3

Table 4. Hydrogen bonds for 4d: Symmetry transformations used to generate equivalent atoms: #1 x,-y, z+$^{1/2}$

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...O(4)#2	0.85(2)	2.01(2)	2.860(8)	173(7)
N(2)-H(2N)...O(1)	0.86(2)	2.01(3)	2.846(7)	165(7)
C(38')-H(38D)...N(2)#3	0.97	2.59	3.28(3)	128.5

Table 5. Hydrogen bonds for 4h: Symmetry transformations used to generate equivalent atoms: #1 -x+1,-y+1,-z+1 #2 x+1,y-1,z #3 -x,-y+1,-z+1

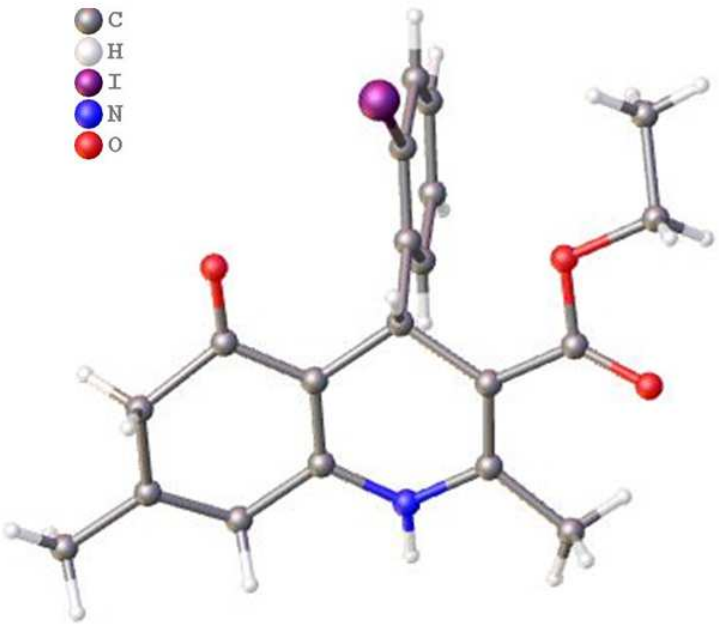


Figure A: Molecular structure and space filled diagram of 4d

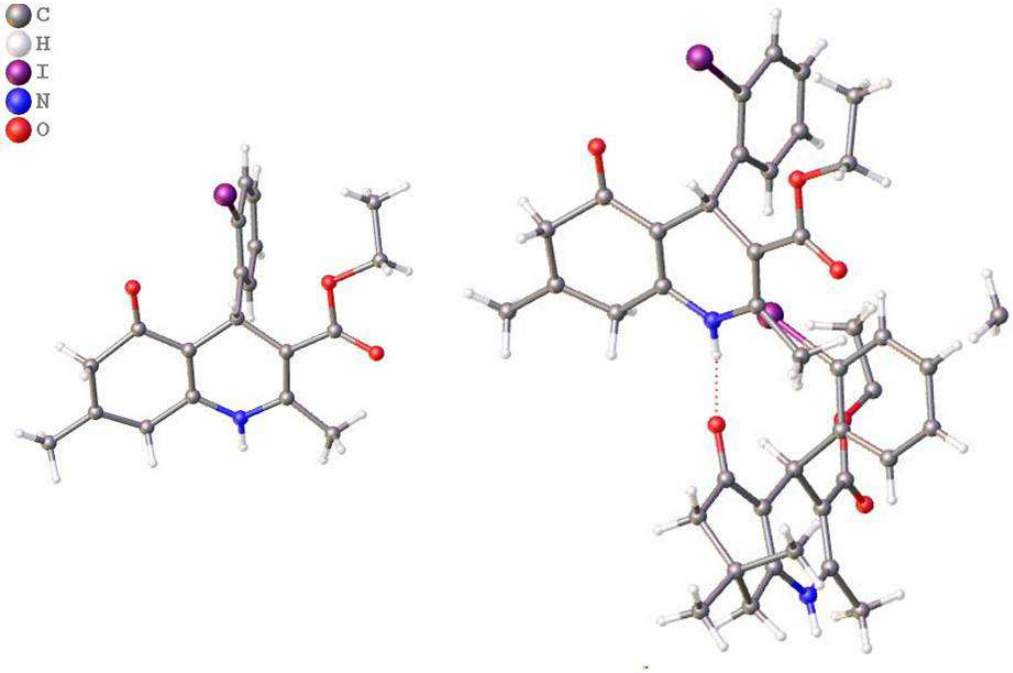


Figure B. Orientation and interactions of 4d

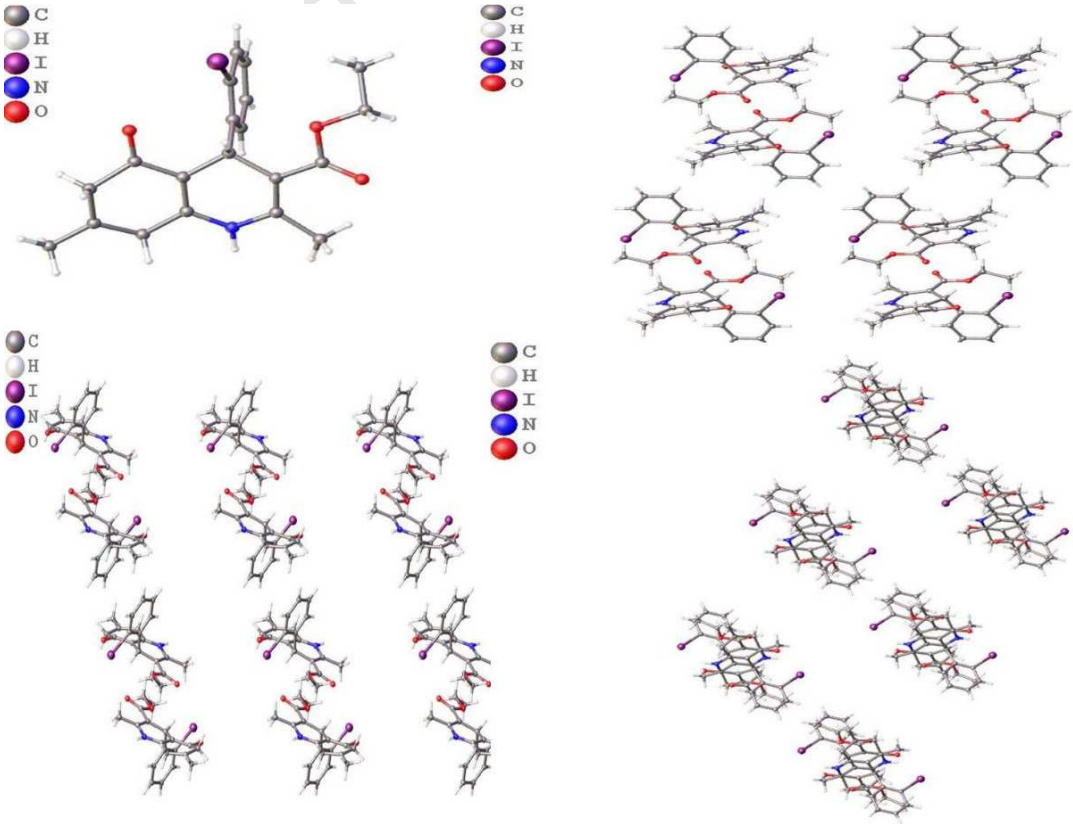


Figure C. Views of the coordination environment of 4d with different axis X, Y, Z (symmetry transformations used to generate equivalent atoms: #1 -x+1,-y+1,-z+1

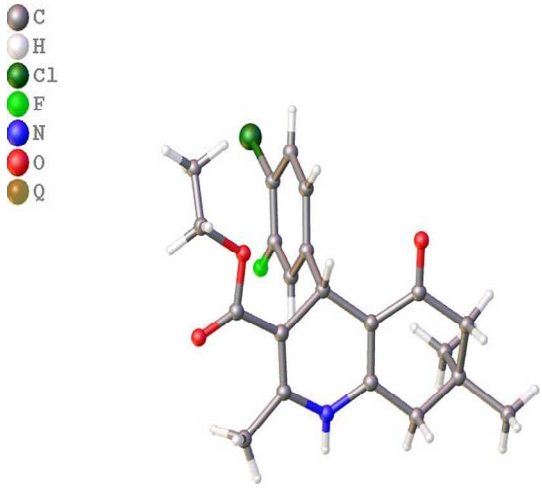


Figure D. Molecular structure and space filled diagram of 4h

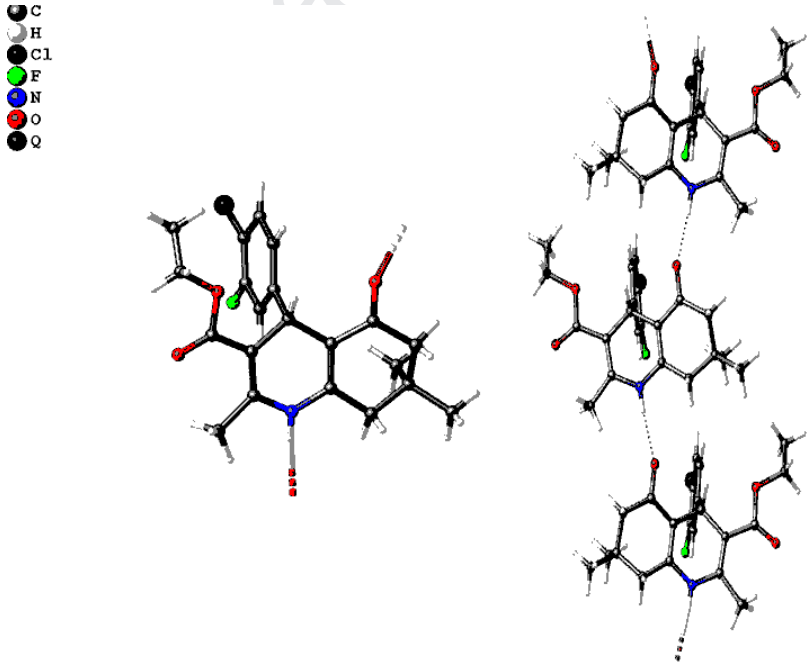


Figure E. Orientation and interactions of 4h

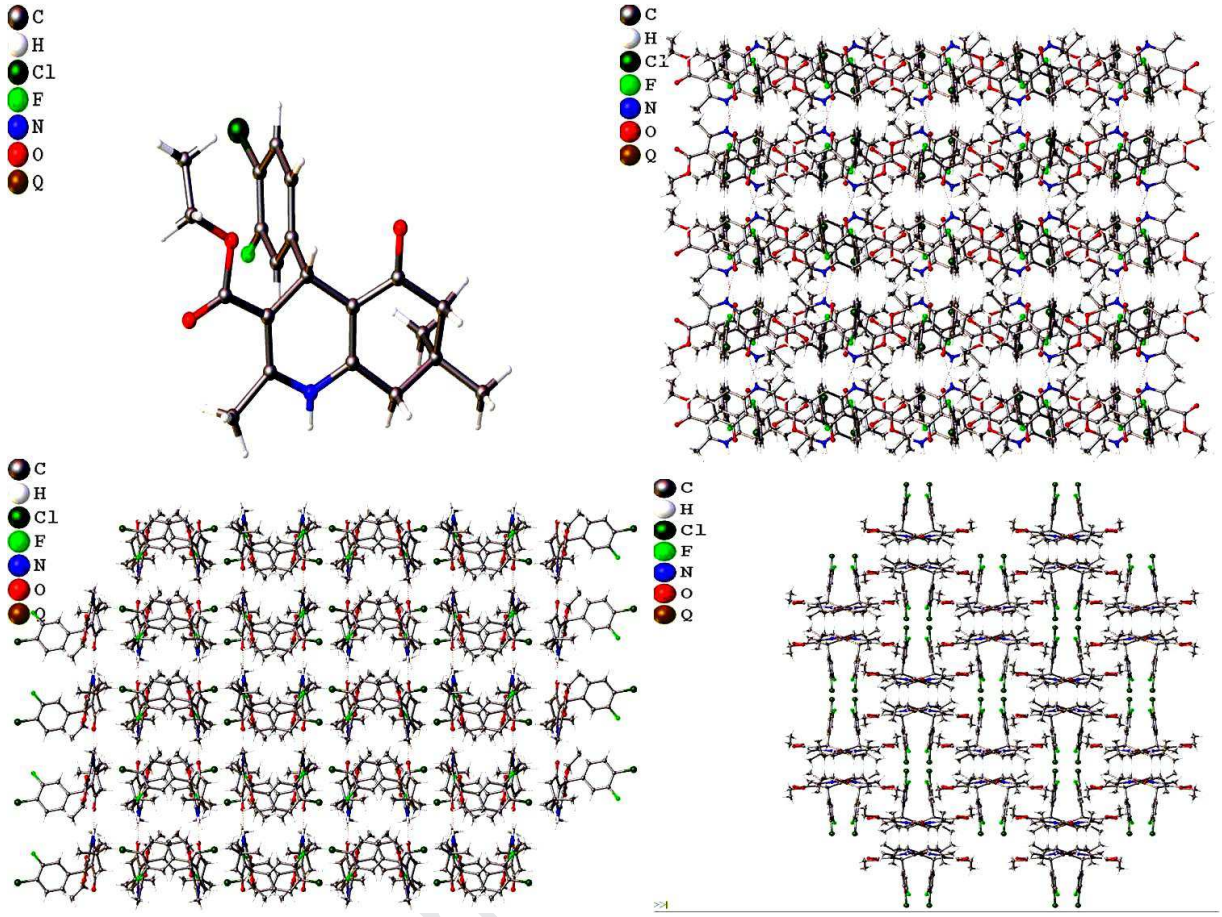


Figure F. Views of the coordination environment of 4h with different axis X, Y, Z (symmetry transformations used to generate equivalent atoms: #1 -x+1,-y+1,-z+1 #2 x+1,y-1,z #3 -x,-y+1,-z+1

The anisotropic thermal parameters of tetrahydroquinoline-3-carboxylic acid ethyl ester (4d and 4h) have been refined for all non-hydrogen atoms. Some of the hydrogen atoms could not be solved, so the analysis was not included. The complete characterization of 4d and 4h crystallographically indicates the formation of the products regioselectively. For the first instance of time, we at this moment report the complete unambiguous characterization of the products. To best of our knowledge there is no precedent for the complete characterization of tetrahydroquinoline-3-carboxylic acid ethyl ester (4d and 4h).

4.1 Crystallographic parameters: bond lengths, bond angles, and torsion angles

In 4d compound, C(1)-C(21), N-H, C-O, C-N, C-Cl, and C-F. Bond lengths are in the range of 1.536(9) and 1.356(8), 1.380(8) and 0.8600, 1.241(7) and 1.458(9), 1.241(7) and 1.458(9), 1.741(11) and 1.332(11) respectively. The bond angles are in the range of C(1)-C(21), 126.7(7)–107.7(5), N-H, 118.3 (5) O(2)-C(16)-C(1), 128.6(8), N(1)-C(3)-C(8) 119.4(6), C(12)-C(13)-Cl(1) 120.3(10) and F(1)-C(12)-C(13)119.6(10). The torsion angles are in the range of C(16)-C(1)-C(2)-C(19 0.3(11), C(9)-C(1)-C(2)-N(1) 5.4(10), C(5)-C(6)-C(7)-O(1) -157.6(6), F(1)-C(12)-C(13)-Cl(1) 2.1(13), F(1)-C(12)-C(13)-C(14) -174.9(9). Therefore, these interactions each comprising of C(1-21) chains form a two dimensional (2D) sheet Inter molecular hydrogen bonding interaction of D-H...A is N(1)-H(1N)...O(1) 174.3 with respect to the Symmetry #1 x,-y,z+1/2.

In 4h compound, C (1)-C (21), N-H, C-O, C-N, and C-I. Bond lengths are in the range of 1.352(9) and 1.527(11), 0.85(2) and 0.86(2), 1.206(8) and 1.458(10), 1.368(8) and 1.380(9), I (1)-C (11) 2.109(7) respectively. The bond angles are in the range of C(2)-C(1)-C(16)119.2(7), C(3)-N(1)-C(2)123.3(6), C(16)-O(3)-C(17) 114.2(7), C(7)-C(6)-H(6B) 108.7 and C(10)-C(11)-I(1)123.4(5). The torsion angles are in the range of C(16)-C(1)-C(2)-C(19)0.3(12), C(9)-C(1)-C(2)-N(1) 5.4(11), C(5)-C(6)-C(7)-O(1)-148.4(7) , I(1)-C(11)-C(12)-C(13)178.2(6). Hence, these interactions each comprising of C(1-21) chains form a two dimensional (2D) sheet. Inter molecular hydrogen bonding interaction of D-H...A were N(1)-H(1N)...O(4)#2, N(2)-H(2N)...O(1) and C(38')-H(38D)...N(2)#3, 173(7), 163(7) and 128.5 with respect to the Symmetry #1 -x+1,-y+1,-z+1 #2 x+1,y-1,z #3 -x,-y+1,-z+1

5. BIOLOGY

5.1 MTT assay

Cell lines used: HepG-2 (Hepatocellular carcinoma cells) and MCF-7 (Human breast adenocarcinoma cells). The anti-cancer activity was tested against the two cell lines- HepG2 and MCF-7. Minimum essential medium (MEM) growth medium augmented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin-B (5 µg/ml) were used to grow the cells in a humidified atmosphere of 5% CO_2 at 37 °C until confluent [13-14]. The cells were trypsinized with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were maintained in 25 mL flat bottles. To determine the antiproliferative effect of the test sample, the cells were seeded in 96-well flat bottom microtitre plates with a density of 1 × 104 cells per well and incubated for 24 h at 37 °C in 5 % CO_2 atmosphere to allow cell adhesion. After 24 h, the medium was removed when partial monolayer was formed. The cells were treated with different concentrations of standard drug (Doxorubicin) and sample compounds for 48 h. Microscopic examination was carried out and observations were recorded at every 24 h. After the treatment, the solutions in the wells were discarded. The wells were then treated with 50 µl of freshly prepared MTT reagent (2 mg/mL prepared in PBS). The plates were shaken gently and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. After 3 h, the supernatant liquid was removed to get the formazan crystals in the wells. Addition of 50 µl of iso-propanol to each well dissolved the formazan crystals. Finally, the optical density was recorded at a wavelength of 540 nm using a Micro-plate reader (Bio-Tek, ELX-800 MS).

The percentage growth was calculated using the following formula:

$$\% \text{ Growth inhibition } = \frac{\text{test absorbance-blank absorbance}}{\text{control absorbance-blank absorbance}} \text{ X } 100$$

The percentage growth concentration from IC_{50} , Concentration of drug required to kill 50% of cells in exponentially growing cultures after a 48 h exposure to the drug for the HepG2, and MCF-7 cell lines. In this study the standard drug was Doxorubicin, the values are obtained and comparable with the values of the compounds **4e** and **4i** which displayed considerable activity **(Table 6).**

Drug/Product	IC_{50} value (µg/ml) on HepG2 cells	Drug/Product	IC_{50} value (µg/ml) on MCF-7 cells
Doxorubicin	1.21 ± 0.05	**Doxorubicin**	1.09 ± 0.03
4a	4.40 ± 0.22	**4a**	4.80 ± 0.16
4b	4.80 ± 0.12	**4b**†	3.40 ± 0.17
4c	4.60 ± 0.25	**4c**†	3.20 ± 0.14
4d	12.50 ± 0.28	**4d**	10.20 ± 0.34
4e†	3.40 ± 0.21	**4e**†	2.80 ± 0.07
4f†	3.20 ± 0.11	**4f**†	3.60 ± 0.21
4g	5.50 ± 0.24	**4g**	4.60 ± 0.22
4h	10.50 ± 0.35	**4h**	7.20 ± 0.80
4i†	3.80 ± 0.09	**4i**†	2.60 ± 0.07

†active

Table 6. *In vitro* anticancer activity of quinolines 4a−4i on HepG2 and MCF-7 Human cancer cell lines

5.1.1 Protein preparation

X-ray crystal structure of ER (PDBID:2IOK) was retrieved from the RCSB protein data bank. Atomic overlaps from the X-ray structure was removed. Auto dock tools were used to prepare

the ER by removing ligand, water molecules, non standard residues and alternate residues. Later polar hydrogens were added to the ER in standard orientation without optimization and Gasteiger charges were added using MGL tools (http://mgltools.scripps.edu/).

5.1.2 Ligand preparation

All the synthesized 3D structures were drawn by Chem Draw software. Drug doxorubicin was retrieved from the complex PDBID 1I1E. Using Argus lab all the drawn structures including doxorubicin were subjected to geometric cleaning and geometry optimization [15]. Gasteiger charges were added and nonpolar hydrogens were merged and rotatable bonds were determined based on the nature of the ligand by using MGL tools.

5.1.3 Grid map generation

After preparing the protein and ligands, the grid maps were generated; spacing was adjusted to 0.500Å to enable ligand binding. Grid dimension is adjusted to 42×64×64 points. AUTODOCK interaction maps were used for docking protocol. Prior to the actual docking run, these maps were calculated by AUTOGRID. For each ligand atom type, the interaction energy between the ligand atom and the receptor was calculated for the entire binding site which is discretized through a grid [16]. The protein was embedded in a 3D grid and a probe was placed at each grid point. Interaction energy of the protein was assigned at each grid point and the affinity grid and electrostatic potential for each atom of the ligand was calculated. Electrostatic interactions were evaluated by interpolation [17].

5.1.4 Docking

Autodock Vina, a docking program was used to evaluate binding affinity of synthesized molecules and doxorubicin with ER [18]. Vina was used to dock the receptor and ligand molecules.

Binding energy of docked complex ER-ligands were evaluated by using empirical free-energy functions and Lamarckian genetic algorithm. The calculated binding free energy (ΔG) is based on the electrostatic, van der Waal's forces, hydrogen bonding and desolvation effects. Finally Vina results were analyzed using the MGL tools.

5.1.5 Results

To know the inhibition interaction of ER by the prepared compounds, the docking tools were used through autodock vina. The binding free energy (ΔG) concept is used to evaluate the binding affinity of protein-ligand complex using docking studies. The negative or low value of binding free energy (ΔG) indicates the strong binding affinity between protein-ligand complex and the ligand in the docking complex is in the most favorable conformation [19-20]. In the present study, comparing the binding affinity between prepared structures and the standard drug using binding free energy (ΔG) in Kcal/mol. Lowest binding free energy (ΔG) docked complex, ER-4e is shown in Figure G and ER-doxorubicin complex is shown in Figure H. Interaction of ER with 4e is shown in the Figure I and J. The binding free energy (ΔG) results are presented in the Table 7, which reflects the binding affinity of the standard and the prepared compound 4e with ER . ER-4e and ER-doxorubicin complexes show binding free energy (ΔG) of −7.9 and −7.3 Kcal/mol respectively. Docking interactions revealed that, doxorubicin interacts with Gln 506 through a hydrogen bond, other amino acids present in the docking sites are: Asn 439, Gln 441, Glu 444, Ala 493, Leu 495 and Arg 503 which plays vital role in binding. Ligand 4e interacts with Thr 347 through hydrogen bond, other amino acids present in the docking site for 4e are: Leu 346, Trp 383, Leu 384, Leu 387, Phe 404, Met 421, Leu 525 and His 524. The analyzed results predict that, **4e** has greater binding affinity among the seven prepared structures and is found to be greater than the drug doxorubicin (Entries 1 and 5).

Entry	Complex	Binding free energy (ΔG) Kcal/mol
1	Doxorubicin	**-7.3**
2	4a	-6.1
3	4b	-6.4
4	4d	-6.3
5	4e	**-7.9**
6	4f	-6.5
7	4g	-6.5
8	4h	-6.9

Table 7. Docking results of the synthesized compounds and doxorubicin drug with ER binding free energy (ΔG) in Kcal/mol

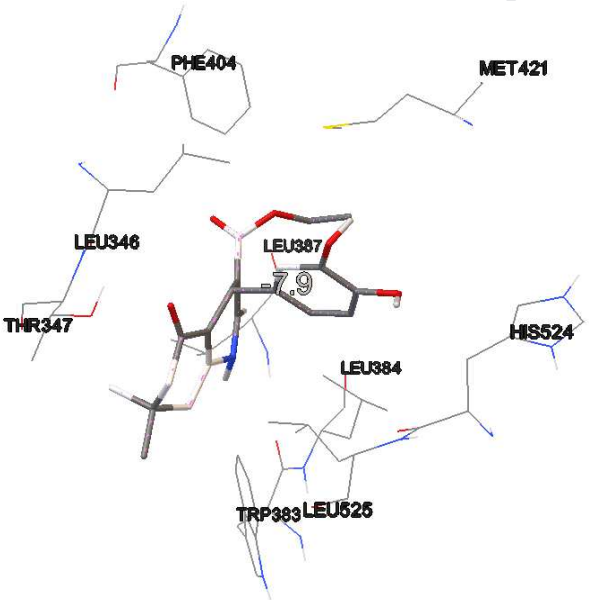


Figure: G

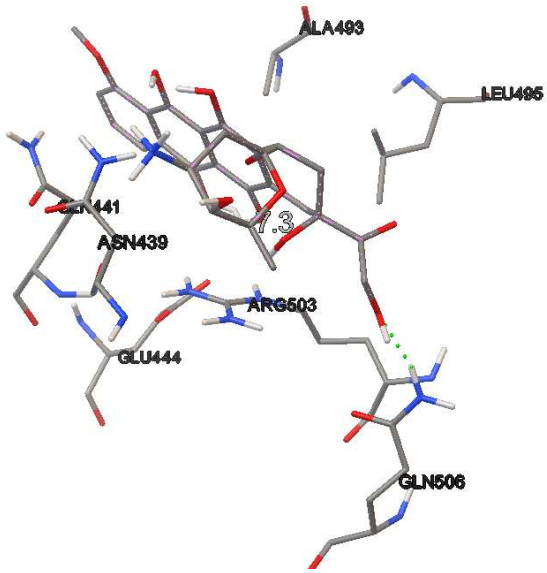


Figure: H

Figure G and H: Stick and wire model of docked complex; **4e** ligand binding at ER docking site and
Stick model showing the hydrogen interaction of doxorubicin with ER.

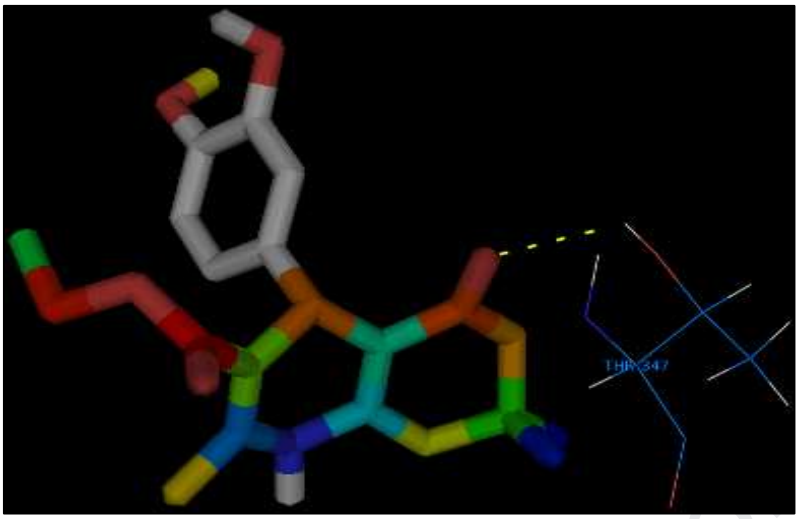


Fig. **I**: Interaction of **4e** with threonine which is present at the docking site ER. Wire: ER; Stick: **4e**.

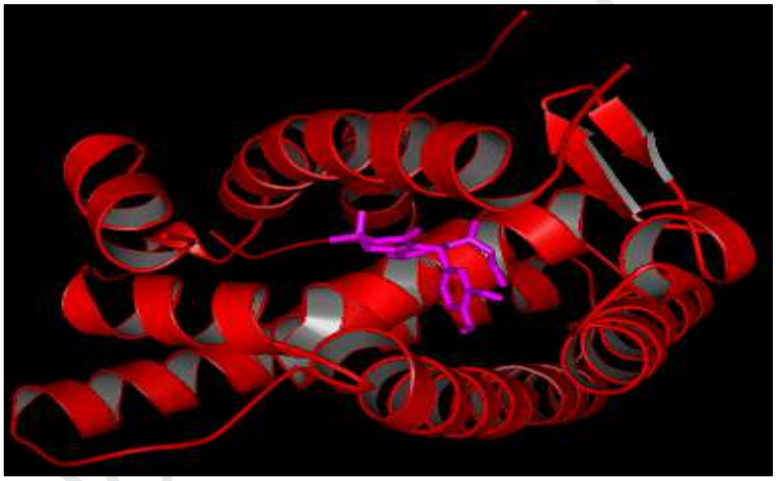


Figure J: Ribbon model of ER-**4e** docked complex showing **4e** present at the docking site of ER. Red ribbon: ER; Magenta stick: **4e**

CONCLUSION

Developed a new green protocol for the synthesis of 4-aryl-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline 3-carboxylic acid ethyl esters by one-pot four-component cyclization reaction of aromatic aldehydes, dimedone, ethyl acetoacetate and ammonium acetate in the presence of a SiO_2-I heterogeneous catalyst using ethanol as a solvent. The reactions are environmentally benign, efficient and mild as it involves the use of a heterogeneous catalyst.

The crystal structures of 4d and 4h were determined by single crystal X-ray diffraction compounds were held by moderate to strong hydrogen bonding interaction of C-H-N, N-H-O and N-H-N respectively and also found that, newly synthesized quinoline derivatives 4e, 4f and 4i showed excellent in vitro anticancer activity towards HepG2 cell lines and 4b, 4c, 4e, 4f and 4i indicated reasonably good activity towards MCF-7 human cancer cell lines, which can be used as lead compounds for developing new potential class of anticancer drugs. Docking results revealed that, 4e compound has more binding affinity towards ER when compared to other seven prepared molecules which is greater than the standard drug: doxorubicin. The obtained in vitro results were correlated with the docking studies.

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Highlights

- Present research work deals with synthesis of one-pot four component route
- Reactions completion is very short time with high yields
- Docking results revealed that, 4e compound has more binding affinity towards ER.
- Structural analysis of synthesized compounds (4d and 4h) by Single Crystal X-ray Diffraction

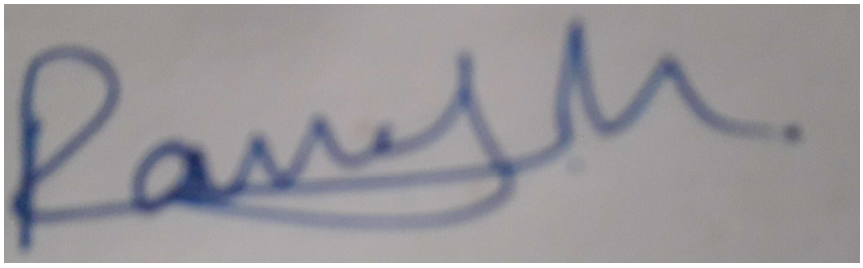
Declaration of interests

- ✓ ☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

No Conflict of interest

Ramesh K B



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