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An Eco-Friendly Synthetic Approach through C(sp3)-H Functionalization of the Viral Fusion "Spike Protein" Inhibitors

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Abstract: The use of organic chemistry to synthesize bio-relevant molecules has been a prime interest of the drug discovery sentients. Recently, our group found 2-styryl substituted quinoline as an anticancer and viral fusion inhibitor through the binding of the gp-41 spike protein. So, we through to synthesize various molecules with an eco-friendly approach. However, various approaches have been reported with the metal, additive, base, and acids to synthesize highly important 2-styrylquinoline from the 2-alkyl quinoline. Still, none of the approaches was reported with the restricted 4-hydroxyquinoline substrate because of their dual nucleophilic nature. The selectivity raises an important issue in this concern substrate. The present protocol overcomes the dual nucleophilic character of the hydroxyl-linked substrate through an optimized eco-friendly sp3 C-H activation approach. The designed protocol for synthesizing gp-41 inhibitors as viral entry inhibitors claimed as metal, acid, and base along with the solvent-free protocol.

Keywords: anti-viral; fusion inhibitors; 4-hydroxyquinoline; solvent-free; metal-free base; acid-free.

1. Introduction

The heterocyclic moiety has been a lucrative asset for the therapeutic regime, with high to low selectivity. The scared electronic distribution and hetero atoms make the pharmacophore an approachable substrate for enzyme-based targets[1, 2]. In the view of medicinal values, various relevant synthetic approaches have been explored with their respective prose[3, 4]. In the ocean of heterocycles, quinoline and its derivatives were found to be our area of interest, which was found with various bioactivity[5, 6]. Recently our group published the activity of 2-styryl quinoline derivatives with 3-ester [7] and their hydrolyzed derivatives, with their high selectivity for the anticancer, because of the favorable physiological condition[8]. The styryl quinolines were also found effective as viral fusion inhibitors (as depicted in Figure 1) in the initial study, so it was thought to extend this study to get the optimized structure with high binding affinity. The interaction of compound 1 with the S2 binding domain (S2 binds with the ACE-II enzyme of the host) confirms the effectiveness of the compounds against the virus[9].

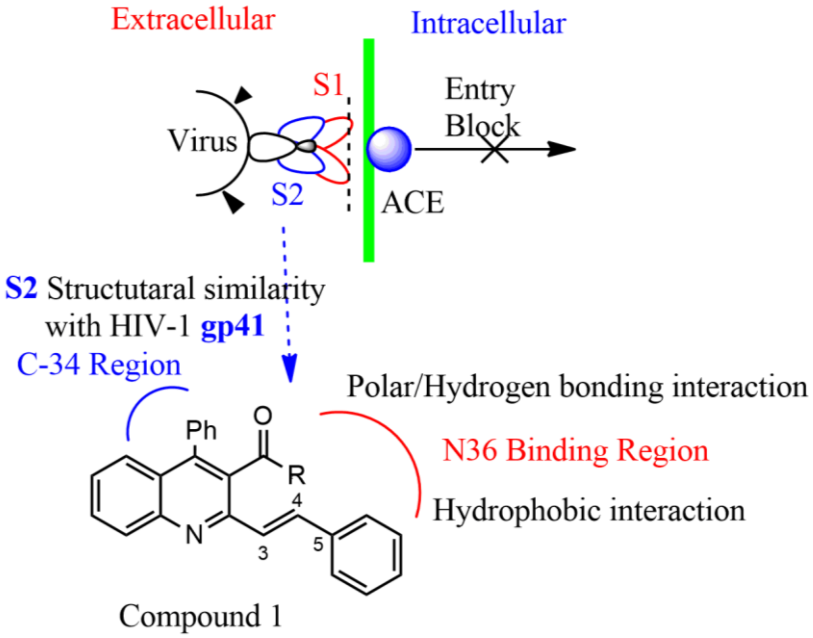


Figure 1. Interaction of Quinoline derivative and gp41.

The infusion of the virus into the cell creates an opportunity to take the resources from the host cell, to grow inside, and transfuse them outside from the host cell to enhance the viral load in the host body. Because of that, the treatment strategy should emphasize the entry or infusion, which will rarely disturb the essential physiology of the host cell and avoid the most concern problem of the anti-viral drug[9].

2. Materials and Methods

2.1. Synthesis and characterization of N-(2-acetyl phenyl)acetamide (9).

To a solution of 2-amino acetophenone 8 (0.10 ml, 0.823 mmol) in DCM has added triethylamine (0.23 ml, 1.65 mmol), and the reaction mixture was cooled to 0 °C. Then acetyl chloride (0.08 ml, 1.23 mmol) was added dropwise and stirred for 3 h at 30 °C. A saturated solution of sodium bicarbonate was added to the reaction mixture. The organic layer was separated and washed with brine (2 X 20 ml), dried over $MgSO_4$, and concentrated in vacuo to give the crude product, which was purified by column chromatography with the use of hexane - EtOAc 98:2 to 90:10 gradient and gave 9 (89 mg, 61%) as white solid. mp 74 - 75 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.24 (s, 3H), 2.68 (s, 3H), 7.11 – 7.14 (m, 1H), 7.54 – 7.58 (m, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 8.75 (d, *J* = 8.0 Hz, 1H), 11.71 (s, 1H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 25.58, 28.64, 120.69, 121.67, 122.30, 131.61, 135.17, 141.01, 169.54, 202.87. MS (ESI): m/z 200 $[M+Na]^+$

2.2. Synthesis and characterization of 2-methyl quinoline-4-ol (5).

To a mixture of N-(2-acetylphenyl)acetamide 9 (200 mg, 1.13 mmol) and crushed NaOH (158 mg, 3.95 mmol) was added anhydrous 1,4-dioxane (8 ml) via syringe and placed in a preheated oil bath at 110 °C. The reaction mixture was stirred for 3 h and then allowed to cool to room temperature. The reaction mixture was then dissolved in methanol and concentrated in vacuo to remove the solvent. The reaction mixture was diluted with a small amount of H_2O then neutralized to pH = 7 with 1 M HCl. Solid precipitated out of the water layer, filtered by Buchner funnel, and concentrated in vacuo to give the crude product, which

was purified by column chromatography (silica gel #230-400, hexane: EtOAc 8:2 v:v) and gave 5 (130 mg, 72%) as a cream-white solid. mp 234 – 235 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 3H), 5.90 (s, 1H), 7.25 – 7.28 (m, 1H), 7.47 – 7.49 (m, 1H), 7.58 – 7.62 (m, 1H), 8.03 (d, *J* = 7.0 Hz, 1H), 11.54 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.91, 108.85, 118.18, 123.14, 124.96, 125.25, 131.89, 140.56, 150.10, 177.20. MS (ESI): m/z 160 $[M+1]^+$

2.3. Synthesis and characterization of (E)-2-styrylquinoline-4-ol (6a).

To a mixture of 2-methyl-4-quinolone 5 (200 mg, 1.257 mmol) and *p*-toluenesulfonamide (215 mg, 1.257 mmol) in neat condition was added benzaldehyde (0.212 ml, 1.88 mmol) and the reaction mixture was refluxed at 140 °C for 24 h. The reaction mixture was cooled to 30 °C, diluted with H_2O and extracted with EtOAc (3 X 20 ml). The combined organic layer was washed with brine (2 X 20 ml), dried over $MgSO_4$ and concentrated in vacuo to give crude product which was purified by column chromatography (hexane-EtOAc 8:2 v:v), and gave 6a (138 mg, 70%) as white solid. mp 284 - 285 °C. IR (KBr, cm^{-1}) ν 3269, 2809, 1634, 1591, 1514, 1140, 960, 754^{1}H NMR (400 MHz, DMSO-d_6) δ 6.53 (s, 1H), 7.21 (d, *J* = 16.6 Hz, 1H), 7.35 – 7.42 (m, 2H), 7.45 – 7.49 (m, 2H), 7.68 – 7.79 (m, 6H), 8.10 (dd, *J* = 8.0, 0.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 107.07, 119.00, 122.25, 124.20, 124.67, 124.95, 127.88, 129.51, 129.97, 132.75, 135.79, 136.37, 140.62, 148.38, 176.16HRMS (ESI) calcd for $C_{17}H_{13}NO$ $[M+H]^+$ 248.1070, found m/z 248.1070

2.4. Synthesis and characterization of (E)-2-(4-fluorostyryl)quinoline-4-ol (6b).

The general procedure for 2, 4-disubstituted quinoline derivatives, the product was obtained as a white solidwith 72% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 282 - 283 °C. IR (KBr, cm^{-1}) ν 3050, 2903, 1586, 1508, 1235, 1172, 963, 756^{1}H NMR (400 MHz, DMSO-d_6) δ 6.37 (s, 1H), 7.07 (d, *J* = 16.7 Hz, 1H), 7.27 – 7.33 (m, 3H), 7.62 – 7.67 (m, 3H), 7.72 – 7.74 (m, 2H), 8.06 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 107.24, 115.86, 116.08, 118.17, 121.91, 123.13, 124.69, 124.97, 129.37, 129.45, 131.97, 133.75, 140.12, 147.00, 161.31, 163.77, 177.17 HRMS (ESI) calcd for $C_{17}H_{12}FNO$ $[M+H]^+$ 266.0976, found m/z 266.0980

2.5. Synthesis and characterization of (E)-2-(2-(trifluoromethoxy)styryl)quinoline-4-ol (6c).

Following the general procedure for 2, 4-disubstituted quinoline derivatives, the product was obtained as a white solidwith 72% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 254 - 255 °C. IR (KBr, cm^{-1}) ν 3434, 3247, 3067, 2934, 1542, 1496, 1156, 958, 756^{1}H NMR (400 MHz, DMSO-d_6) δ 6.34 (s, 1H), 7.14 (d, *J* = 16.7 Hz, 1H), 7.31 – 7.34 (m, 1H), 7.60 – 7.71 (m, 3H), 7.69 – 7.83 (m, 3H), 7.97 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 11.69 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 108.81, 118.83, 123.34, 123.69, 125.23, 125.67, 126.51, 127.09, 128.13, 128.78, 129.74, 130.16, 132.54, 133.58, 140.67, 146.54, 177.60 HRMS (ESI) calcd for $C_{18}H_{12}F_3NO_2$ $[M+H]^+$ 332.0893, found m/z 332.0894

2.6. Synthesis and characterization of (E)-2-(4-(trifluoromethoxy)styryl)quinoline-4-ol (6d).

Following the general procedure for 2, 4-disubstituted quinoline derivatives, the product was obtained as a faint yellow solidwith 74% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 282 - 283 °C. IR (KBr, cm^{-1}) ν 3417, 3258, 3073,

2903, 1507, 1311, 1105, 967, 756^{1}H NMR (400 MHz, DMSO-d_6) δ 6.38 (s, 1H), 7.15 (d, J = 16.7 Hz, 1H), 7.30 – 7.34 (m, 1H), 7.45 (d, J = 8.1 Hz, 2H), 7.65 – 7.71 (m, 3H), 7.80 (d, J = 8.2 Hz, 2H), 8.06 (d, J = 8.0 Hz, 1H), 11.65 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 108.11, 118.70, 119.23, 121.78, 121.95, 123.61, 123.88, 125.21, 125.55, 129.59, 132.47, 133.71, 135.26, 140.65, 147.16, 149.09, 177.64 HRMS (ESI) calcd for $C_{18}H_{12}F_3NO_2$ $[M+H]^+$ 332.0893, found m/z 332.08940

2.7. Synthesis and characterization of (E)-2-(4-(benzyloxy)styryl)quinoline-4-ol (6e).

Following the general procedure for 2, 4-disubstituted quinoline derivatives, the product was obtained as a faint green solidwith 62% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 285 - 286 °C. IR (KBr, cm^{-1}) ν 3122, 3077, 2985, 1589, 1239, 963, 787^{1}H NMR (400 MHz, DMSO-d_6) δ 5.17 (s, 2H), 6.30 (s, 1H), 6.97 (d, J = 16.5 Hz, 1H), 7.09 – 7.11 (m, 2H), 7.27 – 7.36 (m, 2H), 7.39 – 7.42 (m, 2H), 7.46 – 7.48 (m, 2H), 7.57 – 7.65 (m, 5H), 8.04 (d, J = 7.9 Hz, 1H), 11.48 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 107.38, 115.81, 118.59, 120.23, 123.38, 125.18, 125.54, 128.23, 128.41, 128.74, 128.94, 129.33, 132.27, 134.99, 137.26, 140.68, 147.83, 159.78, 177.45 HRMS (ESI) calcd for $C_{24}H_{19}NO_2$ $[M+H]^+$ 354.1489, found m/z 354.1486

2.8. Synthesis and characterization of (E)-2-(2-(thiophen-2-yl)vinyl)quinoline-4-ol (6f).

Following the general procedure for 2, 4-disubstituted quinoline derivatives, the product was obtained as a brown solidwith 65% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 272 - 273 °C. IR (KBr, cm^{-1}) ν 3421, 3095, 2971, 1547, 1324, 1166, 950,759^{1}H NMR (400 MHz, DMSO-d_6) δ 6.35 (s, 1H), 6.85 (d, J = 16.2 Hz, 1H), 7.14 – 7.16 (m, 1H), 7.23 – 7.29 (m, 1H), 7.38 (d, J = 3.3 Hz, 1H), 7.60 – 7.65 (m, 3H), 7.87 (d, J = 16.3 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 106.94, 119.21, 121.79, 123.29, 125.07, 125.66, 128.29, 128.50, 128.87, 129.79, 132.04, 141.17, 141.25, 147.71, 177.06 MS (ESI): m/z 276 $[M+Na]^+$

2.9. Synthesis and characterization of (E)-2-(4-morpholinostyryl)quinoline-4-ol (6g).

Following the general procedure for 2, 4-disubstituted quinoline derivatives, thproduct was obtained as a white solidwith 69% yield after purification by column chromatography(hexane-EtOAc 8:2 v:v).mp 284 - 285 °C. IR (KBr, cm^{-1}) ν 3422, 3247, 2923, 1584, 1347, 1235, 1119, 927, 760^{1}H NMR (400 MHz, DMSO-d_6) δ 3.22 (t, J = 4.8 Hz, 4H), 3.75 (t, J = 4.8 Hz, 4H), 6.29 (s, 1H), 6.91 (d, J = 16.9 Hz, 1H), 7.02 (d, J = 8.9 Hz, 2H), 7.25 – 7.32 (m, 1H), 7.53 – 7.64 (m, 5H), 8.04 (d, J = 8.0 Hz, 1H), 11.42 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 47.97, 66.42, 107.05, 115.07, 118.52, 118.60, 123.23, 125.16, 125.56, 126.29, 128.96, 132.15, 135.32, 140.70, 148.09, 152.08, 177.30 HRMS (ESI) calcd for $C_{21}H_{20}N_2O_2$ $[M+Na]^+$ 355.1417, found m/z 355.1416.

3. Results and Discussions

In this development process, various substitutions were tried with the concern pharmacophore. However, the styryl functionalization of 4-hydroxy quinoline was most difficult because of their non-selective or least reactivity of the substrate. The need for a favorable method was in the scientific arena to motivate us to develop A Versatile method even

for the least reactive substrate. However, many reported methods are available for the sp^3 C-H bond activation-based link, as depicted in Figure 2[10-23].

The reported method was tried with the concern substrate (compound 5) for the styryl link, as depicted in Figure 3. All metal-mediated reagents were tried first because of the lone pair chelating capability of the metal extramural activity for an extended substrate. The metal-based reaction protocol could not find sufficient to oxidize into styryl form; the starting material (5) remains intact for 24 hr. in all the metal-based reported methods.

In search of an efficient reaction protocol with the substrate (5), the literature reported with the metal-free condition was adopted. The conversion yield with the acetic anhydride (Table 1, entries 2 and 3) was unsatisfactory because of the low conversion rate. The reaction condition with NaOAc was reported (Table 1, Entry 4) with the substrate (2), while in the case of hydroxyl substrate (5), the starting material (5) remains intact for 24 hr. (Scheme 1). A little hope came up with the use of *p* toluene sulfonamide with toluene as per the reported protocol; however, further investigation was needed to get the best result.

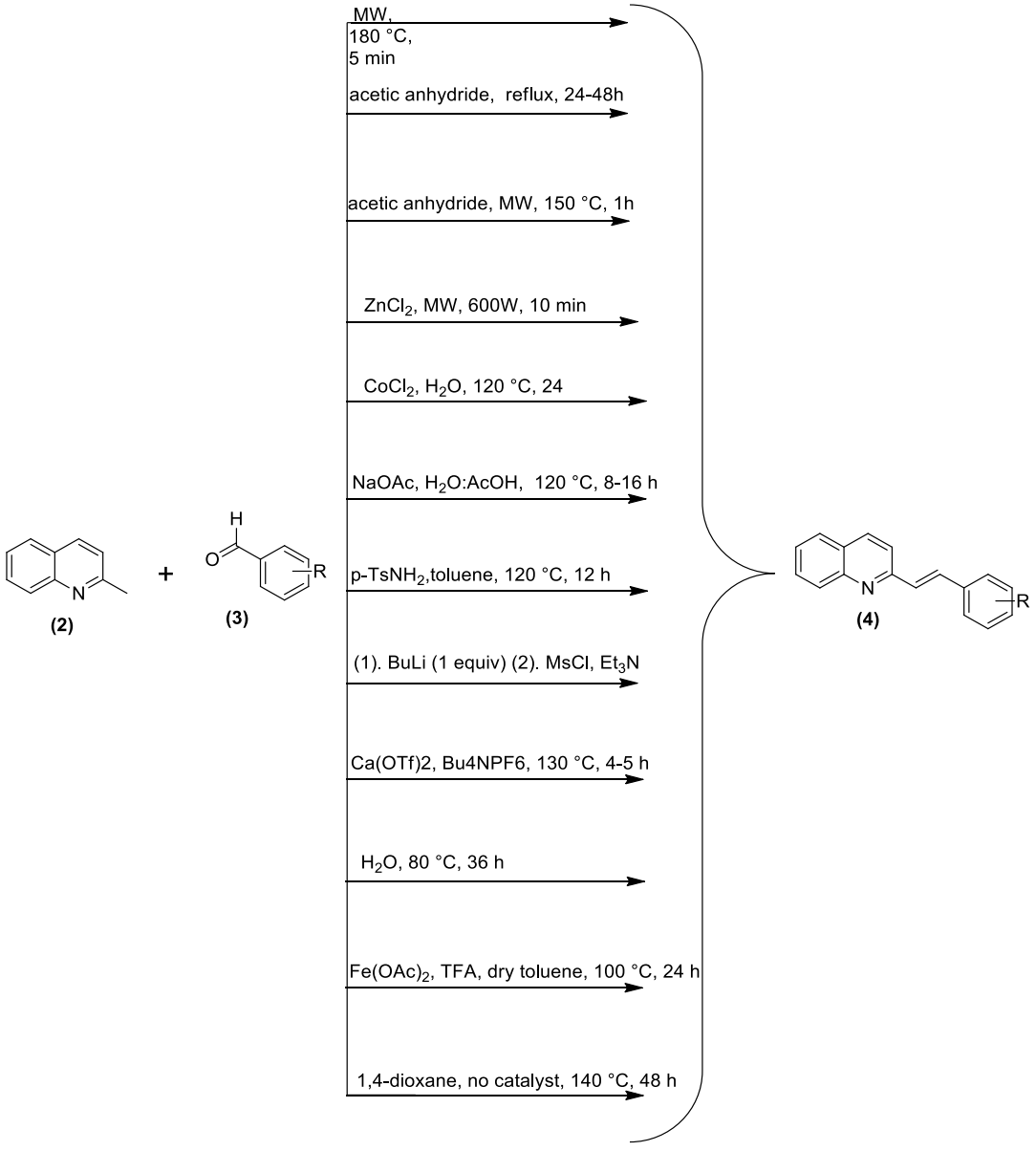


Figure 2. Reported method for synthesis of quinoline [10-23].

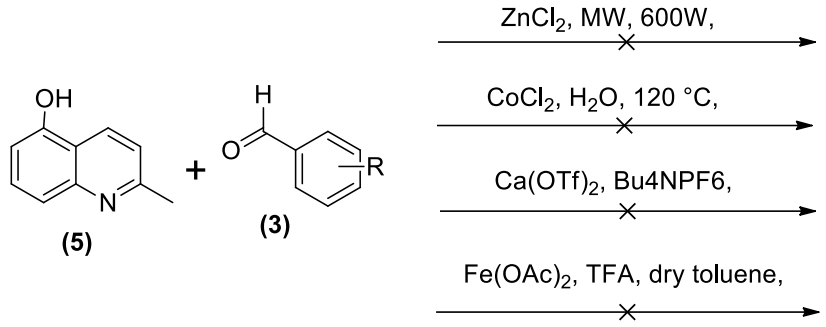
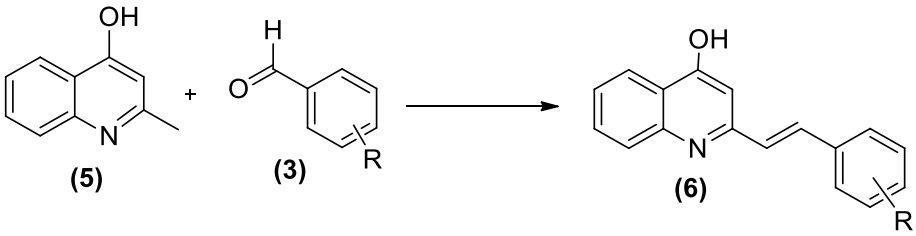


Figure 3. Reported metal-based protocol for sp3 C and H functionalization [9-20].



Scheme 1. Condensation of aldehyde**.**

Table 1. Reaction and conditions for 2-substituted-4-hydroxyquinoline derivatives.

Reagents	Conditions	Yields[a] (%)
Neat, MW	180 °C, 5 Min	No product[9]
Acetic anhydride, reflux	150 °C, 48 h	3010
Acetic anhydride, MW	150 °C, 1 h	No product[11]
NaOAc, H_2O: AcOH	120 °C, 24 h	No product[14]
p-toluenesulfonamide, toluene	120 °C, 12 h	39[15]

[a]yield calculated after chromatographic isolation.

The slow reaction and low yield were overcome by using para-toluene sulfonamide, which came up with hope for the synthesis of the concerned product (6), which allowed optimizing the reaction protocol. The low yield was anticipated because of the dual nucleophilic character of the substrate (5), where the hydroxyl group (Nu^1) was dominating the nucleophilic character of alkene (Nu^2), as depicted in Figure 4.

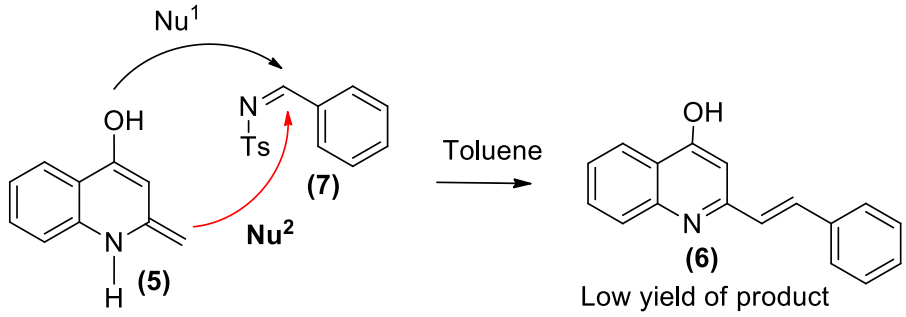
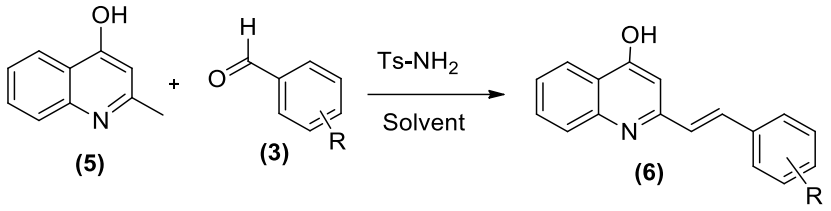


Figure 4. A reported method for synthesis of quinoline.

In search of an efficient solvent system with the reagent (Table 2 and Scheme 2), This was found suitable, with the least reactive substrate (5). Various solvents were tried, like non-polar, polar protic, and aprotic; among them, polar protic was founds to be the least activator than the polar aprotic(Table 2 Entry 7) and non-polar (Table 2, Entry 1 and 2). The reaction was found to be sensitive to water content; water use was detrimental to the optimized reagent. (Table 2, Entry 3). The exiting result was observed when the solvent was eradicated from the

system; it suggested the dilution factor played a role in this reaction condition. The optimized condition was subjected to other parameters.



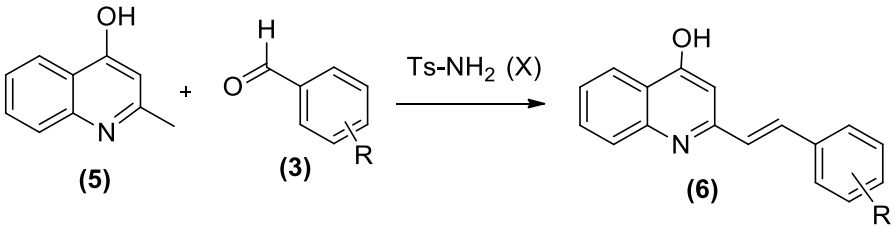
Scheme 2. Synthesis of 4-hydroxy-2-styrylquinoline.

Table2. Reaction and conditions for 2-substituted-4-hydroxyquinolinederivatives**.**

S.No.	Solvent	Yields[a] (%)
1.	Hexane	42
2.	Dry Toluene	46
3.	H2O	No product
4.	EtOAc	20
5.	Ethanol	15
6.	Methanol	17
7.	DMF	40
8.	Neat	67

[a]yield calculated after chromatographic isolation**.**

The detrimental effect of the solvent leads us to think to optimize the amount of *p*-toluene sulphonamide; however, the exponential growth in the reaction rate was observed till the amount of reagent was enhanced to the 1.5 molar ratios. The amount of-*p*-toluene sulfonamide was optimized with the previously found best combination of reagents as per the below-given Table 3and Scheme3.



Scheme 3. Synthesis of 4-hydroxy-2-styrylquinoline.

Table3. Reaction and conditions for 2-substituted-4-hydroxyquinoline derivatives.

S.No.	X (mmol)	Yieldsa (%)
1.	1	65
2.	1.2	70
3.	1.3	78
4.	1.4	83
5.	1.5	81
6	2	83

[a]yield calculated after chromatographic isolation.

The reason for the high yield was further analyzed, and it was anticipated that after the 1 molar quantity, it was making a hydrogen bond with the hydroxyl group, which helped to reduce the nucleophilic nature of the hydroxyl group. The hydrogen bonding protection was the reason for the nucleophilic addition reaction of alkene, as depicted in the below-given Figure5.

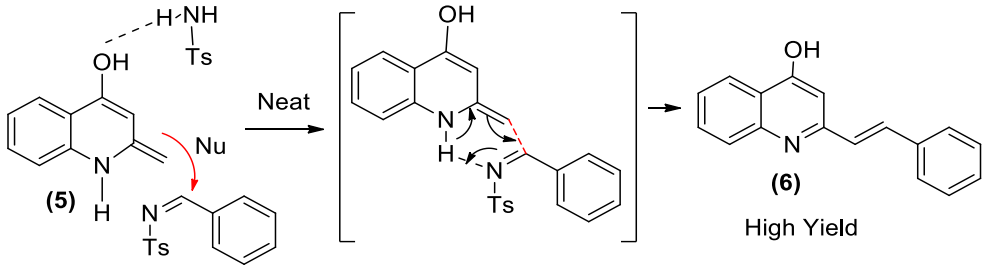


Figure 5. Effect of *p*-toluene sulfonamide in the optimized reaction.

The process of the reaction was anticipated to start with the nucleophilic nature of the quinoline parts if a strong nucleophile is present like 4-hydroxy derivatives (5). Otherwise, the pi-electron of alkene (because of the high energy electron) takes part in the reaction. The reaction pattern was also confirmed with the fluoro substituted counterpart(6d) because of their high yield and lower reaction time. The feasibility of the reaction with the electron-deficient counterpart gives evidence of nucleophilic addition reaction, as depicted in Figure 6.

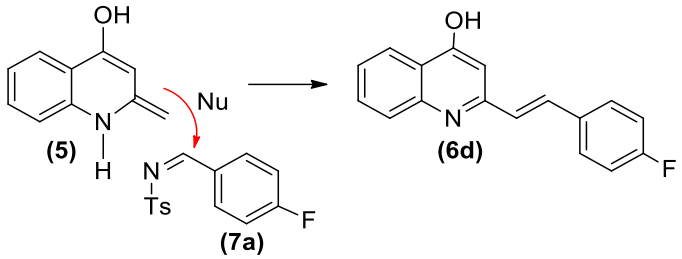


Figure 6. Effect of fluoro substitution in the reaction time and yield.

^{13}C NMR spectroscopy data of the compound 6d revealed the presence of nine protons in the aromatic region and seventeen carbons in the range of δ value 177-107 ppm, and each carbon at the δ value 107.24, 115.87, 116.08, 118.18, 121.91, 123.14, 124.70, 124.97, 129.37,129.45,131.98,133.75,140.12,147.01,161.31,163.77,177.18.^{19}FNMR(Figure7)spectroscopy data revealed the presence of one fluorine group at the δ value -111.6.

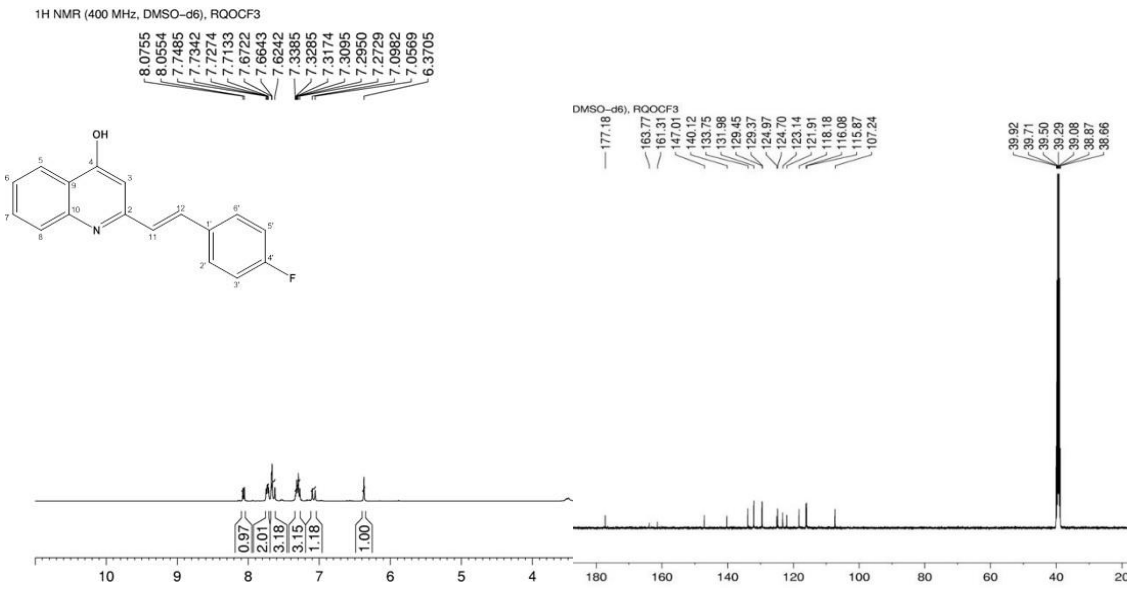


Figure 7. C^{13} and ^{1}H NMR of compound 6d.

3.1. 2DNMR.

Organofluorine compounds show heteronuclear coupling,i.e.:

1. Coupling between fluorine and hydrogen
2. Coupling between fluorine and carbon

The coupling between fluorine and carbon is unique in ^{13}C NMR, which is a reliable criterion for determining fluorine-containing compounds. In ^{13}C NMR, peak splitting at δ value 161.31 (C-4'), 115.87 (C-3' and C-5') and 129.37 (C-2' and C-6') confirms one bond C-F coupling (J = 248.7 Hz), two bond C-F coupling (J = 21.6Hz) and three bond C-F coupling (J = 8.3 Hz) respectively *i.e.* $^{1}J_{(C\text{-}F)}$ = 248.7 Hz; $^{2}J_{(C\text{-}F)}$= 21.6 Hz; $^{3}J_{(C\text{-}F)}$ = 8.3 Hz.

The ^{1}H- ^{1}H COSY correlations H-5 with H-6, H-6 with H-7 and H-5, H-7 with H-8, H-11 with H-12, H-2' with H-3' and H-5' with H-6'showed the connectivity of adjacent proton (Figure 8). The proton at δ 7.07 and 7.64 was assigned as H-11 and H-12, respectively.

The δ value of all carbons of the molecule was assigned based on HSQC correlation (Figure 8). The H-3 was correlated with carbon at δ 147.01 (C-2) 177.18 (C-4), 124.70 (C-9), and 121.91 (C-11) while, H-5' was correlated with quaternary carbon at δ 161.31 (C-4') and 131.98 (C-1'). H-6'was correlated with quaternary carbon at δ 133.75 (C-12), 161.31 (C-4'), and 131.98 (C-1'). Olefinic proton (H-12) at δ 7.64 was correlated with δ 121.91 (C-11), 147.01 (C-2) and 131.98 (C-1') while the proton (H-11) at δ 7.07 correlated with carbon at δ 133.75 (C-12), 107.24 (C-3), 147.01 (C-2) (Figure 8). Protons of quinoline rings (H5, H6, H-7, and H-8) were also assigned based on their correlations.

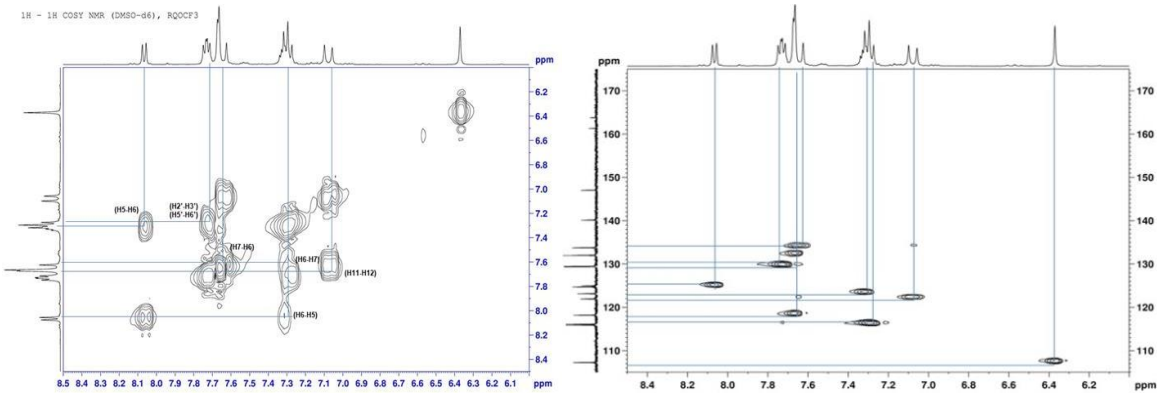


Figure 8. COSY and HSQC spectrum of compound 6d**.**

To rationalize the dual role of *p*-toluene sulfonamide and the substrate (2), where hydroxyl group was not present to interfere, was done, which led to the point that the conversion rate is not proportional to this the amount of *p*-toluene sulfonamide. The conclusion was drawn from this experiment that the amount of *p*-toluene sulfonamide does not interfere with the alkene, so for substrate (2), the yield will be fixed, while in the case of a substrate (5) it is anticipated the high yield is because of the hydroxyl protection nature due to their nucleophilicity (Figure 9).

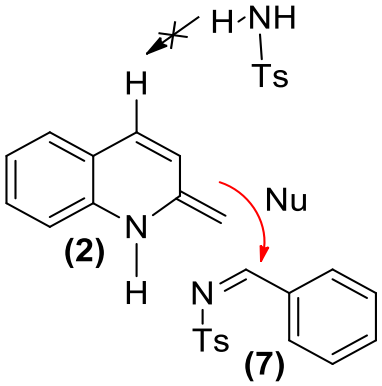


Figure 9. Effect of *p*-toluene sulfonamide quantity in a substrate (2).

3.2. Intermolecular competition among substrates 2 and 5.

Further, the analysis of substrate with (5) or without hydroxyl group (2) for the reactivity was performed with the equimolar quantity of coupling partner (7) to analyze the interfering nature of the OH group. The reaction was started with the equimolar quantity of substrates 2 and 5 in one reaction vessel with the substrate. The process was analyzed, and the product formation (4) was found with the substrate (2). However, the substrate (5) remains intact because of the least reactive or interfering nature (Figure 10). It justifies the dual nucleophilic character of the substrate (5) and the low yield of the product through the reported methods.

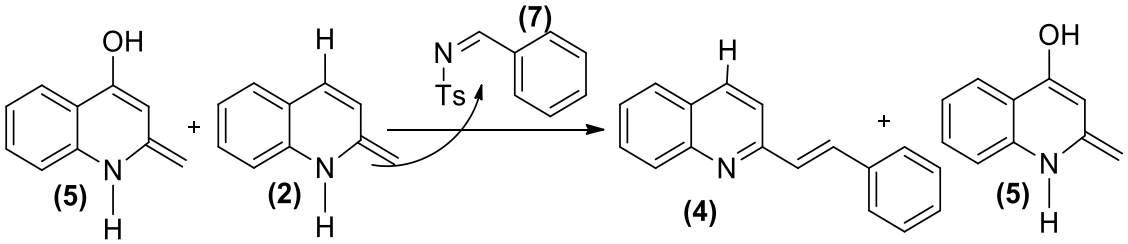
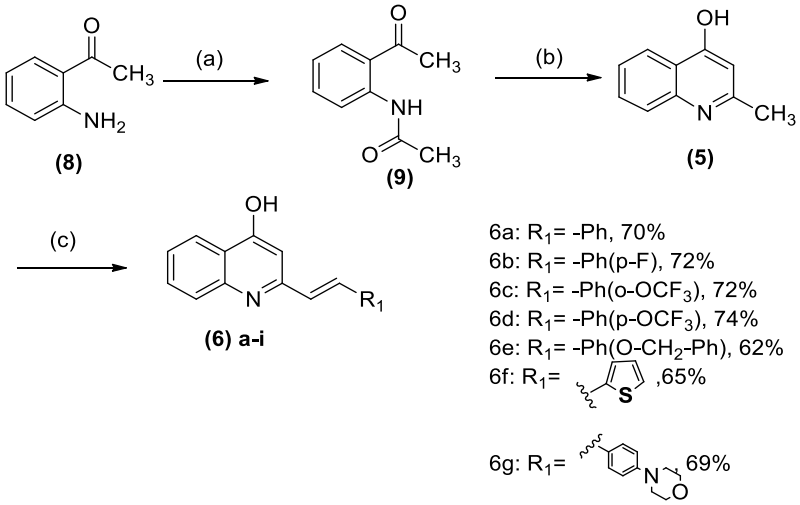


Figure 10. Intermolecular competition of substrate with the *p*-toluene sulfonamide**.**

The above experiments suggest that hydroxyl as a nucleophile has a detrimental effect in all cases, except where the excess quantities of the *p*-toluene sulfonamide were used. It gives a positive note regarding the additional hydrogen bond from the *p*-toluene sulfonamide and their direct effect on the product yield. Despite the unique character of the protocol, the present protocol was found with some additional green chemistry values with the following character:solvent-free,metal-free,base-free, acid-free.

The optimized protocol was used to synthesize various 4-hydroxy2-styrylquinoline derivatives.

To find the substrate scope of the optimized protocol, the coupling of sp^3 C and H of compound 5 was done by refluxing with *p*-toluene sulfonamide in neat condition at 140°C to afford 2-substituted-4-hydroxyquinoline derivatives (6a-g) in 62-74% yield as described below (Scheme 4).



Scheme 4. Synthesis of 2, 4-disubstituted quinoline derivatives (6a-g)**.**

Reagents and conditions: (a) CH_3COCl, triethylamine, DCM, rt, 3 h, 61% (b) NaOH, dioxane, 110°C, 3 h, 72% (c) aromatic aldehyde, *p*-toluene sulfonamide, neat, 140°C, 28-45 h, 62-74.

3.3. Plausible reaction mechanism.

2, 4-disubstituted quinoline derivatives were synthesized using previously reported methods [9-20] which were modified as illustrated in Scheme 2. The plausible mechanism for the reaction of 2-methyl quinoline with benzaldehyde in the presence of *p*-$TsNH_2$ is outlined in Figure 11. In the first step, benzaldehyde reacts with *p*-$TsNH_2$and yields *N*-tosylaldimine (7) *in situ*. Subsequently, enamine intermediate (7a), which is formed from 2-methyl quinoline, reacts with *N*-tosylaldimine (7) to yield intermediate (10) through a cyclic transition state (7a). At last, desired product 6,i.e., 2-styrylquinoline, was obtained from intermediate 10 via elimination.

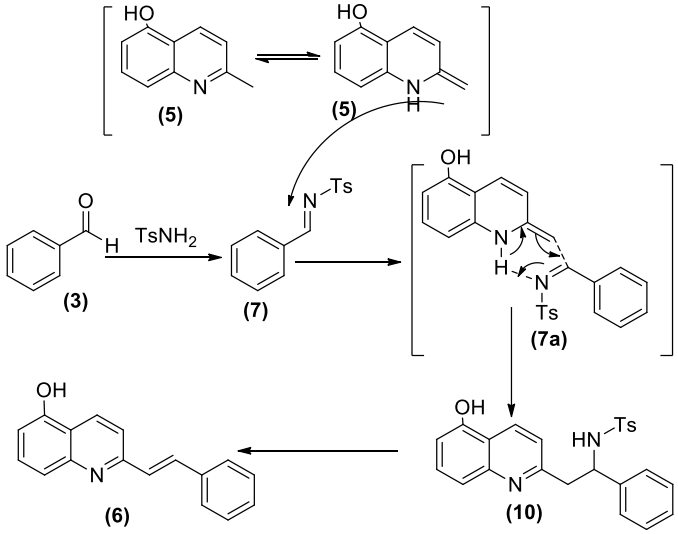


Figure 11. Plausible mechanism for the reaction of 2-methyl quinoline with benzaldehyde in the presence of *p*-$TsNH_2$

3.4.In-silico evaluation.

3.4.1. Docking.

All the synthesized derivatives were docked on various validated HIV target proteins to determine the mechanism of action so that further molecules could be designed to act/bind on the same target.

3.4.2. Preparation of proteins.

The hydrophobic region in gp41 was exposed by removing one C34-helix from six-helix bundles for docking studies [24].

3.4.3. Preparation of ligands.

The ligand was prepared using the AutoDock tool, while its structure was made using ChemBio Draw Ultra software. Merging of non-polar hydrogens, addition of Gasteiger charges, and assignment for autodock atom type were done while energy minimization was achieved with MOPAC using the PM3 method. [25]

3.5. Quantitative estimation of drug-likeness (QED).

QED was calculated for all the synthesized compounds. These compounds were ranked as per their drug-likeliness value, which was assessed using Lipinski's rule of five (RO5), the

first rule based on the physicochemical properties of the compounds. The RO5 also inspired various other concepts for drug-likeliness, such as the rule of three, Ghose, Hughes, Gleeson, and Veber rule of drug-likeness, which failed to provide absolute drug-likeliness. Quantitative estimate of drug-likeness (QED) is a much recently proposed method that is based on the concept of desirability.

3.6. In-silico prediction of absorption, metabolism, and toxicity properties.

AdmetSAR software was used to calculate important properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) [26-27].

3.7. Molecular docking studies.

These designed and synthesized 2, 4-disubstituted quinoline derivatives have been docked for the active site of integrase, reverse transcriptase, and GP41 using AutoDock4. Rilpivirine, approved by USFDA in 2011 as a reverse transcriptase inhibitor, was used as a control. These derivatives have shown moderate to good binding to GP41 for the anti-HIV activity, as shown in Table 4[28, 29].

Table 4. Molecular docking studies of 2, 4-disubstituted quinoline derivatives 6a-g**.**

Compounds	GP41
6a	-6.30
6b	-6.42
6c	-6.27
6d	-7.14
6e	-6.15
6f	-6.58
6g	7.19

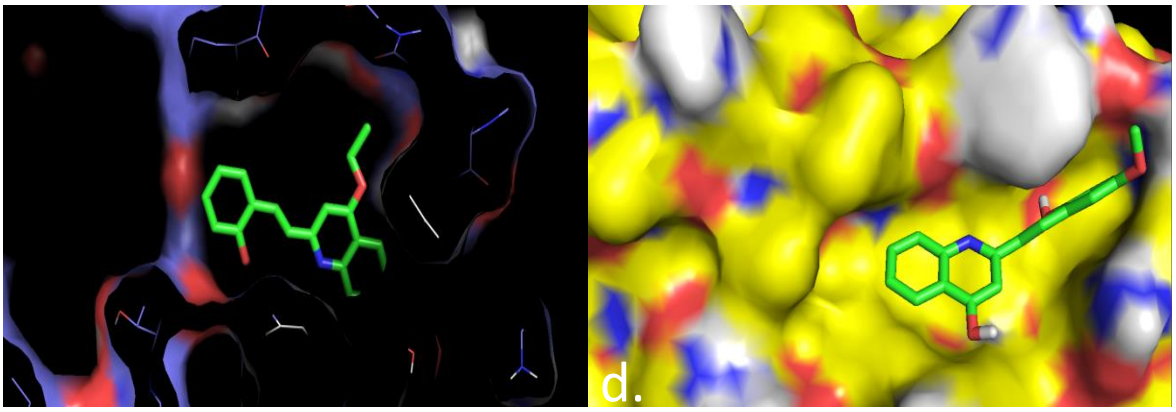


Figure 12. Binding interaction of compounds 6d and 6g with GP-41**.**

3.8. Quantitative estimation of drug-likeness (QED) prediction of 2, 4-disubstituted quinoline derivatives.

The QED values of 2, 4-disubstituted quinoline derivatives 6a-g were found to be in a range of 0.34 - 0.75, as shown in Table 5.

Table 5. Predicted QED of 2, 4-disubstituted quinoline derivatives 6a-g**.**

Compounds	QED
6a	0.747
6b	0.747
6c	0.569
6d	0.569
6e	0.498

Compounds	QED
6f	0.753
6g	0.616

3.9. In-Silico prediction of absorption, metabolism, and toxicity properties of 2, 4-disubstituted quinoline derivatives 6a-g.

Absorption, metabolism, and toxicity of 2, 4-disubstituted quinoline derivatives were predicted by using the AdmetSAR tool. All derivatives were found to be non-toxic and non-carcinogenic except 6g as depicted in Table 6.

Table 6. *In-silico* prediction of absorption, metabolism, and toxicity of 2, 4-disubstituted quinoline derivatives6a-g.

		6a	6b	6c	6d	6e	6f	6g
Absorption	BBB	+	+	+	+	+	+	+
	HIA	+	+	+	+	+	+	+
	Caco-2permeability	+	+	+	+	+	-	+
	P-GPS	NS	NS	NS	NS	NS	NS	S
	P-GPI	NI	NI	NI	NI	NI	NI	NI
Metabolism	CYP450 2C9substrate	NS	NS	NS	NS	NS	NS	NS
	CYP450 2D6substrate	NS	NS	NS	NS	NS	NS	NS
	CYP450 3A4substrate	NS	NS	NS	NS	NS	NS	NS
	CYP450 1A2inhibitor	I	I	I	I	I	I	I
	CYP450 2C9inhibitor	NI	NI	I	NI	NI	I	NI
	CYP450 2D6inhibitor	NI	NI	NI	NI	NI	I	NI
	CYP450 2C19inhibitor	NI	NI	I	I	I	I	NI
	CYP450 3A4inhibitor	NI	NI	NI	NI	NI	NI	NI
Carcinogenicity & Mutagenicity	AMESToxicity	NT	NT	NT	NT	NT	NT	NT
	Carcinogen	NC	NC	NC	NC	NC	NC	NC

BBB: Blood-brain barrier; HIA: Human intestinal absorption; p-GPS: P-glycoprotein substrate; p-GPI: P-glycoprotein inhibitor; NT: Nontoxic; T: Toxic; NC: Non-carcinogenic.

4. Conclusions

The eco-friendly and versatile approach was finalized with the analysis of various parameters; moreover, it was found suitable for 4-hydroxyquinoline moiety, which was found fit for gp-41, a similar protein of the virus surface with *in-silico* favorable ADME toxicity studies also. Further study can be extended to get the viral inhibitors, which contain spike protein. The future of the reactions protocol led us to the broad spectrum anti-viral agent. Moreover, the design protocol avoids the use of metal, solvent, and any base or acid, which was present in most of the reported protocol as per the reported protocol. The various 2, 4-disubstituted quinoline derivatives were synthesized by modifying the ancillary aromatic ring with a wide range of substituents, including small groups such as chloro, fluoro, trifluoromethyl, trifluoromethoxy, hydroxyl, aromatic groups like phenyl, benzyloxy, and some heteroaromatic rings like morpholine. The present protocol will play an important role in the functionalization of the reactive as well as the least reactive quinoline nucleus. The present protocol will help design and synthesize the hydroxy-linked quinoline to get the broad spectrum anti-viral as fusion inhibitors, strengthening the medical science for the future viral attack.

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Conflicts of Interest

There is no conflict of interest.

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