Mapana Journal of Sciences
2018, Vol. 17, No. 2, 53-59
ISSN 0975-3303|https://doi.org/10.12723/mjs.45.6

Synthesis, Characterization and Biological Activity of Qinoline bound Imidazoles

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Abstract

8-Hydroxyqunoline derivatives were synthesised by treating 5-chloromethyl-8-hydroxy quinoline with imidazole and characterized using NMR and mass spectrometry. 2-mercaptoimidazole derivatives of 8-hydroxy quinoline were also synthesized and characterized. One of the compounds in this series has been tested for dengue activity along with other series of compounds, but did not show any activity against dengue virus.

Keywords: Dengue virus, 8-hydroxy quinoline, imidazole, 2-mercaptoimidazole

1. Introduction

Dengue fever is estimated by WHO to cause 50-100 million infections yearly with ~22,000 deaths, mostly among children[1]. Dengue virus (DENV) is responsible for an acute febrile illness, with occasional serious complications from thrombocytopenia and plasma leakage that leads to pleural effusion, and hypovolemic shock[2].

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In a previous study, high throughput screening (HTS) using West Nile Virus (WNV) protease as the target and identified inhibitors with chemically diverse scaffolds has been performed. One group of compounds has the 8-hydroxyquinolines (8-HQ) as the chemical scaffold with different substituents of 8-HQ ring (7-position). Based upon initial findings some 8-Hydroxy quinoline derivatives have been found to have antidengueactivities[3,4]. These series of compounds were mannich bases of 8-hydroxy quinolone with different amines. This prompted us to replace amines with imidazole and 2-mercapto imidazole, synthesize the compounds and study their biological activity.

The present study deals with the synthesis and characterization of quinoline bound imdiazoles and study of the anti-dengue property.

2. Experimental methods

2.1 Chemicals and apparatus

8-Hydroxyquinoline, 5-chloro-8-hydroxyquinoline and paraformaldehyde were obtained from S.D.Fine chemicals (LR grade). Imidazole was obtained from Chem-labs Ltd. India.Isopropyl-1-thio-β-D-galactopyranoside was obtained from Sigma Aldrich Ltd. The fluorogenic tetra-peptide substrates, Bz-Nle-Lys-Arg-Arg-AMC was custom synthesized by NeoBioScience (Cambridge, MA). AMC was procured from Anaspec, Inc (Fremont, CA).

The melting points of the compounds were recorded on BUCHI melting point instrument B-540. ^{1}H NMR spectra were recorded (in DMSO-d_6/$CDCl_3$) on A 400MHz NMR spectrometer. A BUCHI B-540 spectrometer was used to record mass spectra. Thin layer chromatography (TLC) was used to monitor the progress of the reactions and purity of the products. Appropriate solvent systems were used as eluents.

Procedure for the synthesis of 5-(1*H*-imidazol-1-ylmethyl)quinolin-8-ol hydrochloride (5)

To a solution of 5-(chloromethyl) quinolin-8-ol hydrochloride (4) (0.021mole) in 50ml of *N,N*- dimethylformamide (DMF) was added imidazole (1) (0.043mole) under stirring. The mixture was heated to 90-95°C for 6 hours. The solvent is distilled off completely. The

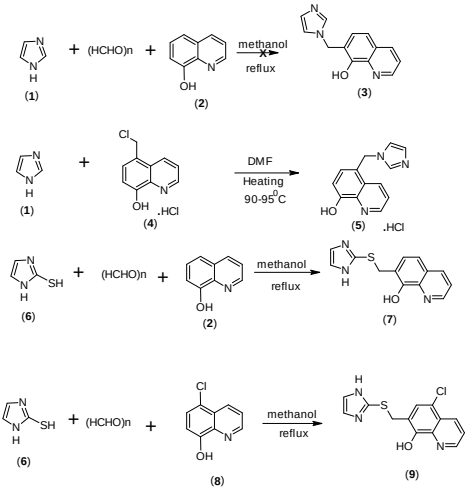
obtained residue is treated with chloroform and heated to 60°for 30minutes. The product 5-(1*H*-imidazol-1-ylmethyl)quinolin-8-ol hydrochloride (5) was filtered and dried in the oven at 70-75°C for 4 hours, m.p. 262-264 °C.

Procedure for the synthesis of7-[(1*H*-imidazol-2-ylsulfanyl)methyl]quinolin-8-ol (7)

To a solution of8-hydroxy quinoline (2) (0.015mol) in 25ml of methanol was added paraformaldehyde (0.015mol) and 2-mercaptoimidazole (0.015mol) under stirring. The mixture is heated to 50-55° for 6 hours. The solvent is distilled off completely. To the residue obtained added diethylether. The precipitated product7-[(1*H*-imidazol-2-ylsulfanyl)methyl]quinolin-8-ol (7) was filtered and dried at 70-75°C for 4hrs, m.p. 218-220°C.

Procedure for the synthesis of5-(chloromethyl) quinolin-8-ol hydrochloride (4):

A mixture of 8-hydroxyquinoline (0.05mol), 8ml. of concentrated hydrochloric acid and 37% formaldehyde (0.05mol) was treated with hydrogen chloride gas for 90min. The product was filtered and dried to get 5-(chloromethyl) quinolin-8-ol hydrochloride (4), m.p. 283 °C (decomposition).



Scheme 1: Synthesis of quinoline bound imidaoles

2.2 Preparation of samples for biological studies

The DENV2 NS2BH-NS3pro expression plasmid encoding the protease precursor used in this study contains the hydrophilic domain of NS2B cofactor (45 amino acids) and the N terminal NS3 protease domain (185 amino acids) separated by the two naturally occurring amino acids, QR, at the P2 and P1 positions, respectively at the N-terminus of the DENV2 NS2B-NS3pro cleavage site. The construction of the expression plasmid encoding the DENV2 NS2B-QR-NS3pro in pQE30 vector (Qiagen) has been described previously[7]. The expression and purification of the DENV2 NS2B-QR-NS3pro were achieved as follows. E. coli Top 10 F′ cells (Invitrogen, Carlsbad, CA) were transformed with the plasmid pQE30 DENV2 NS2B-NS3. Bacterial growth was monitored at 37° C upto an optical density of0.6 at 600 nm. Isopropyl-1-thio-β-D-galactopyranoside (1mM) induced cells were incubated at 16° C for 20 hours. Cells were separated by centrifugation (5000 x *g*) for 15 min at 4° C, washed with 50 mMTris-HCl (pH 7.5) and 200 mMNaCl buffer solution, centrifuged (5000 x *g*) for 15 min at 4° C. The pellet was re-suspended with 50 mM HEPES (pH7.0), 500 mMNaCl, 0.5% Triton X-100, and 0.05 mg/ml lysozyme; then, incubated 30 min on ice. Cells were disrupted by sonication (pulse 15 seconds in 1 min) for 20 min, and centrifuged (15000 x *g;* 30min;4° C). Centrifugate was collected and incubated for 1 h at 4°C with Talon resin (BD Bioscience, San Jose, CA), which was pre-equilibrated in 50 mM HEPES (pH7.0), 500 mMNaCl (buffer A). The affinity resin was centrifuged at 400 x *g* for 10 min at 4° C, washed with buffer A and packed into the column. The column was washed with 15 ml of 10 mM imidazole in buffer A, and eluted with 500 mM imidazole in buffer A. The concentrated fractions were determined by Bradford Reagent (Thermo Scientific), pooled, and dialyzed against 1 liter of 50 mMTris-HCl, pH 7.5, 300 mMNaCl, and 40% Glycerol. The aliquots were stored at ─80° C until use. The compounds were dissolved in dimethylsulfoxide (DMSO) to yield 25 mM stocks, and stored at -20° C.

3. Results and Discussion

Imidazole (**1**) is reacted with 8-hydroxyqinoline (**2**) and paraformaldehyde under different conditions. This reaction did not give the expected product 7-(1*H*-imidazol-1-ylmethyl) quinolin-8-ol (**3**). Further 8-hydroxyquinoline (**2**) is reacted with paraformaldehyde in aqueous hydrochloric acid as reported in litrature[5] to get 5-(chloromethyl)quinolin-8-ol hydrochloride (**4**)**.** This was reacted with imidazole to give 5-(1*H*-imidazol-1-ylmethyl) quinolin-8-ol hydrochloride (**5**)**.** Compound **(5)** has been screened for anti-dengue activity. 2-Mercptoimidazole **(6)** was reacted with 8-hydroxyqinoline **(2)** and paraformaldehyde to get7-[(1*H*-imidazol-2-ylsulfanyl) methyl]quinolin-8-ol **(7)**. The same reaction has been repeated with 5-chloro-8-hydroxyquinoline (**8**)to afford 5-chloro-7-[(1*H*-imidazol-2-ylsulfanyl)methyl]quinolin-8-ol (**9**)**,** scheme 1. 2-Mercaptoimidazole (**6**) required for the work is prepared according to the reported procedure[6].

The newly synthesized compounds were characterised by using ^{1}H NMR and mass spectral data and the spectral details of selected compounds are given below (**4, 5** and **7**)

5-(1*H*-imidazol-1-ylmethyl) quinolin-8-ol hydrochloride (5): Pale yellow crystalline solid; Yield(78%); Melting point:262-264˚C. ^{1}H NMR(400MHz, DMSO-d6) δ_H(ppm): 9.93 (s, hydroxyl O-H, 1H), 8.86(m, quinoline, 1H), 8.54(m, quinoline, 1H), 7.75(s, imidazole, 1H), 7.60(q, *J*=4.12Hz, quinoline, 1H), 7.36(d, *J*=7.88Hz, quinoline, 1H),7.10(t, *J*=0.96Hz, imidazole-H, 1H), 7.05(d, *J*=7.84Hz, quinoline, 1H), 6.85(s, imidazole-H, 1H), 5.56(s, CH_2, 2H); $(m+1)^+$ 226.

7-[(1*H*-imidazol-2-ylsulfanyl)methyl]quinolin-8-ol (7): Yellow crystalline solid; Yield(82%); Melting point:218-220˚C. ^{1}H NMR(400MHz, DMSO-d6) δ_H(ppm): 12.20(s, Imidaole N-H, 1H), 9.94(s, hydroxyl O-H, 1H), 8.86(m,quinoline, 1H), 8.69(m, quinoline, 1H), 7.58(q, *J*=4.12Hz, quinoline, 1H), 7.44(d, *J*=7.84Hz, quinoline, 1H), 7.06(d, *J*=7.84Hz, quinoline, 1H), 6.84(m,imidazole-H, 1H), 6.78(m, imidazole-H, 1H), 5.47(s, CH_2, 2H); $(m+1)^+$258.

5-(chloromethyl)quinolin-8-ol hydrochloride (4): Yellow solid; Yield(82%); Melting point:283˚C(dec). ^{1}H NMR(400MHz, DMSO-

d6) δ_H(ppm): 9.08(m, quinoline, 2H), 8.02(q, quinoline, 1H), 7.69(d, quinoline, 1H), 7.50(d, quinoline, 1H), 4.15(s, CH_2, 2H).

3.1 Biological Activity

In this study, the efficacy of compound (**5**) along with a number of 8-HQ derivatives with different substitutions at the 7-position of the 8-HQ ring by using an in vitro protease assay has been evaluated. Among which one of the compound inhibited the viral protease in vitro as well as DENV2 replication in mammalian cells expressing the DENV2 Renilla luciferase (Rluc) reporter replicon. We describe here the construction and characterization of the DENV2 Rluc reporter replicon expressing baby hamster kidney (DENV2-Rluc-BHK21Rep) cell line.Results are presented in Table 1 and Fig. 1**.** Compound(**5**) did not show any activity against dengue virus.

Table 1. Percent Inhibition of DENV2 NS2BH-NS3pro cleaving tetrapeptide substrate at 37 °C

Compounds	Percent Inhibition		Compounds	Percent Inhibition	
	mean	SD		mean	SD
Aprotinin	98.85566	1.297374	KN6815	7.081533	11.75169
KN15	-6.925985	14.25354	KN6905	-7.84115	8.602437

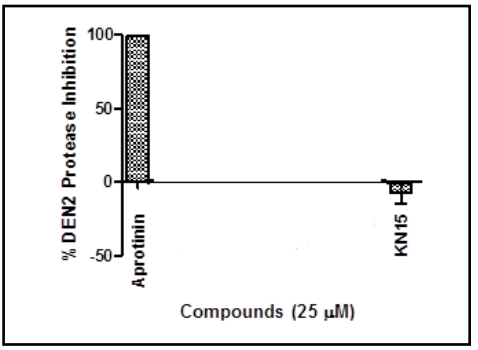


Figure 1: Protease inhibition of 5-(1*H*-imidazol-1-ylmethyl)quinolin-8-ol hydrochloride (5)

4. Conclusion

Three quinoline bound imdiazoles were synthesized and characterized by ^{1}H NMR and mass spectral data successfully. Among synthesized compounds one compound (**5**) has been screened for antidengue property. It did not show activity against dengue virus.

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