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Synthesis and study the anti-proliferative effect of new series of 1*H*-imidazo[4,5-*c*]quinoline derivatives in MCF-7 (human breast cancer) cells

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Abstract : New series of 2-(4-(7-fluoro-8-aryl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile derivatives of biological interest have been synthesized from commercially available 2-amino-5-bromo-4-fluorobenzoicacid with good yield (70%). Compounds 2-(4-(7-fluoro-8-phenyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile (7a) and 2-(4-(7-fluoro-8-(4-iodophenyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile (7b) were also screened for anti-proliferative effect on MCF-7 (human breast cancer cell line). Among them compound 7a showed moderate inhibition compared to control.

Keywords : 1*H*-Imidazo[4,5-*c*]quinoline, 2-amino-5-bromo-4-fluorobenzoicacid, MCF-7, anti-proliferation assay.

Introduction

Breast cancer is the most prevalent form of cancer in women world over. MCF-7 cells are useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogens, in the form of estuarial, via estrogens receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogens receptor (ER) positive control cell line. In breast cancer cells containing oestrogen receptors both oestrogens and growth factors can stimulate proliferation, invasion and the secretion of a number of proteins. In the drug discovery activities, imidazo[4,5-*c*]-quinoline derivatives can be considered a privileged ATP-site-directed kinase lead structure and also were found to act as inhibitors of the PI3K/PKB-pathway[1], selective dipeptidyl peptidase IV (DPP-4)[2] and TNF-α (tumor necrosis factor-α) suppressor[3]. Even though 1*H*-imidazo-[4,5,*c*]quinoline derivatives acted as a protein kinase inhibitor[4], in order to increase the rate of inhibition, fluorine was introduced in 7-position. Fluorine, being the second smallest substituent that meets the steric requirements at enzyme receptor sites, the presence of fluorine increased lipid solubility and thereby improved the pharmacological activity. This may be due to the enhancement of the rate of absorption and transport of drugs *in vivo* and the aided hydrophobic interaction between drugs and binding sites on receptor or enzyme[5]. Furthermore, bromine was replaced by electron donating aryl group at 8-position of 1*H*-imidazo[4,5,*c*]quinoline to study the toxicity of MCF-7 cells.

Experimental

5-Bromo-4-fluoro-2-(2-nitrovinylamino)benzoic acid (**1**) :

^{1}H NMR (300 MHz, DMSO-d_6) : δ 13.0 (1H, d), 8.20 (1H, d), 8.0 (1H, m), 7.95 (1H, d), 6.90 (1H, d); LCMS (M-1) 304.

6-Bromo-7-fluoro-3-nitroquinolin-4-ol (**2**) :

^{1}H NMR (300 MHz, DMSO-d_6) : δ 13.1 (1H, br s), 9.25 (1H, s), 8.41 (1H, d), 7.61 (1H, d); LCMS (M+1) 286.6.

6-Bromo-4-chloro-7-fluoro-3-nitroquinoline (**3**) :

^{1}H NMR (300 MHz, DMSO-d_6) : δ 9.42 (1H, s), 8.76 (1H, d), 8.22 (1H, d); LCMS (M+1) 304.9.

2-(4-(6-Bromo-7-fluoro-3-nitroquinolin-4-ylamino)phenyl)acetonitrile (**4**) :

^{1}H NMR (300 MHz, DMSO-d_6) : δ 10.15 (1H, br s), 9.05 (1H, s), 8.95 (1H, d), 7.95 (1H, d), 7.3 (1H, d), 7.1 (1H, d), 4.05 (1H, s); LCMS (M+1) 400.8.

2-(4-(3-Amino-6-bromo-7-fluoroquinolin-4-ylamino)-phenyl)acetonitrile (**5**) :

^{1}H NMR (300 MHz, DMSO-d_6) : δ 8.64 (1H, s), 8.05 (2H, m), 7.76 (1H, d), 7.1 (1H, d), 6.52 (1H, d), 5.42 (2H, br s), 3.84 (1H, s); LCMS (M+1) 370.9.

2-(4-(8-Bromo-7-fluoro-1H-imidazo[4,5-c]quinolin-1-yl)phenyl)acetonitrile (**6**) :

^{1}H NMR (300 MHz, $CDCl_3$) : δ 9.4 (1H, s), 8.62 (1H, s), 8.1 (1H, d), 7.82 (1H, d), 7.76 (2H, m), 7.6 (1H, d), 4.3 (2H, s); LCMS (M+1) 380.8.

2-(4-(7-Fluoro-8-aryl-1H-imidazo[4,5-c]quinolin-1-yl)phenyl)acetonitrile (**7**) :

To a suspension of compound **6** (0.15 g, 0.39 mmol) in toluene, ethanol and water (1 : 1, 8 mL), sodium carbonate (0.104 g, 0.98 mmol) was added. The reaction mixture was degasified for 15 min. To the degasified reaction mixture was added boronic acid (0.43 mmol) and $Pd(PPh_3)_4$ (0.04 g, 0.03 mmol) under nitrogen atmosphere. The reaction mixture was then heated at 100 °C for 10 h (monitored by TLC) and cooled to room temperature and then diluted with water and extracted with ethyl acetate (3 × 20 ml). Combined ethyl acetate layer was washed with brine, dried over anhydrous sodium sulphate and filtered. Solution was evaporated and the residue was purified by silica gel column chromatography using chloroform-methanol (95 : 5) mixture as eluent. Pure product was obtained with the yield of 70%.

2-(4-(7-Fluoro-8-phenyl-1H-imidazo[4,5-c]quinolin-1-yl)phenyl)acetonitrile (**7a**) :

Pale yellow solid, m.p. 190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) : δ 9.36 (1H, s), 8.62 (1H, s), 8.01–8.04 (1H, d), 7.85–7.87 (2H, d), 7.72–7.74 (2H, d), 7.39–7.47 (5H, m), 4.25 (2H, s); ^{13}C NMR (400 MHz, DMSO-d_6) : δ 159.25, 156.78, 146.25, 144.81, 137.03, 135.59, 134.45, 133.72, 133.15, 129.90, 128.82, 128.57, 128.35, 127.95, 122.05, 122.01, 118.99, 114.95, 114.73, 114.48, 22.14; LCMS (M+1) 379.

2-(4-(7-Fluoro-8-(4-iodophenyl)-1H-imidazo[4,5-c]quinolin-1-yl)phenyl)acetonitrile (**7b**) :

Pale yellow solid, m.p. 210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) : δ 9.36 (1H, s), 8.62 (1H, s), 8.01–8.04 (1H, d), 7.84–7.86 (2H, d), 7.73–7.75 (2H, d), 7.66–7.68 (2H, d), 7.41–7.43 (1H, d), 7.32–7.34 (1H, d), 4.27 (2H, s); ^{13}C NMR (400 MHz, DMSO-d_6) : δ 159.01, 156.54, 146.43, 144.84, 137.06, 135.52, 133.81, 133.62, 131.83, 130.54, 130.51, 129.94, 127.92, 121.95, 119.1, 115.05, 114.84, 114.46, 22.2; LCMS (M+1) 505.

Results and discussion

Chemistry :

New series of 2-(4-(7-fluoro-8-aryl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile derivatives **7(a,b)** of biological interest were synthesized by the reaction between commercially available 2-amino-5-bromo-4-fluorobenzoic acid and nitro methane followed by cyclisation and chlorination using phosphorous oxy chloride. Resulting 6-bromo-4-chloro-7-fluoro-3-nitroquinoline was treated with 4-cyano benzyl amine prepared in house from 4-nitrophenylacetonitrile by reduction using Raney nickel in presence of hydrogen to give (**4**). This on further reduction with Raney nickel and hydrogen gave the reduced compound 2-(4-(3-amino-6-bromo-7-fluoroquinolin-4-ylamino)phenyl)acetonitrile (**5**). This was cyclised using triethylorthoformate via known procedure[4] followed by Suzuki cross coupling reaction with phenyl boronic acid and *p*-iodo phenyl boronic acid using tetrakis (triphenylphosphine) palladium(0) with the yield of 70% shown in Fig. 1. The synthesized compounds **7a** and **7b** were characterized by ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and LCMS.

Anti-proliferation assay on MCF-7 cells :

The anti-proliferation assay of the samples was studied by MTT using MCF-7 (breast cancer cell). 100 µL of 2 × 10^4 cells were seeded in 96-well culture plate. The plate was incubated at 37 °C in a humidified 5% CO_2 atmosphere. After 1 day, different concentration of compounds **7** (**a** and **b**) dissolved in dimethyl sulfoxide (DMSO) were added to plate and kept for 24 h, 48 h and 72 h. The media was removed and incubated with fresh culture medium containing 100 µL of MTT for 4 h in darkness. Then, the unreacted dye was removed and formazan salt was dissolved in 100 µL DMSO and the amount was determined by measuring the optical density (OD) at 540 nm. The relative cell viability was determined by the amount of MTT converted into formazan salt. Cell viability in presence of test compound was quantified compared to that of the cell control (Fig. 2). Among the two compounds, **7a** exhibited a significant inhibition at the highest tested concentration of 20 µM at 24 h. The percentage inhibition was found to be 14%. Whereas at 48 h and 72 h, 10 and 20 µM concentrations of **7a** showed 15% and 33% inhibition, and 21% and 35% inhibition

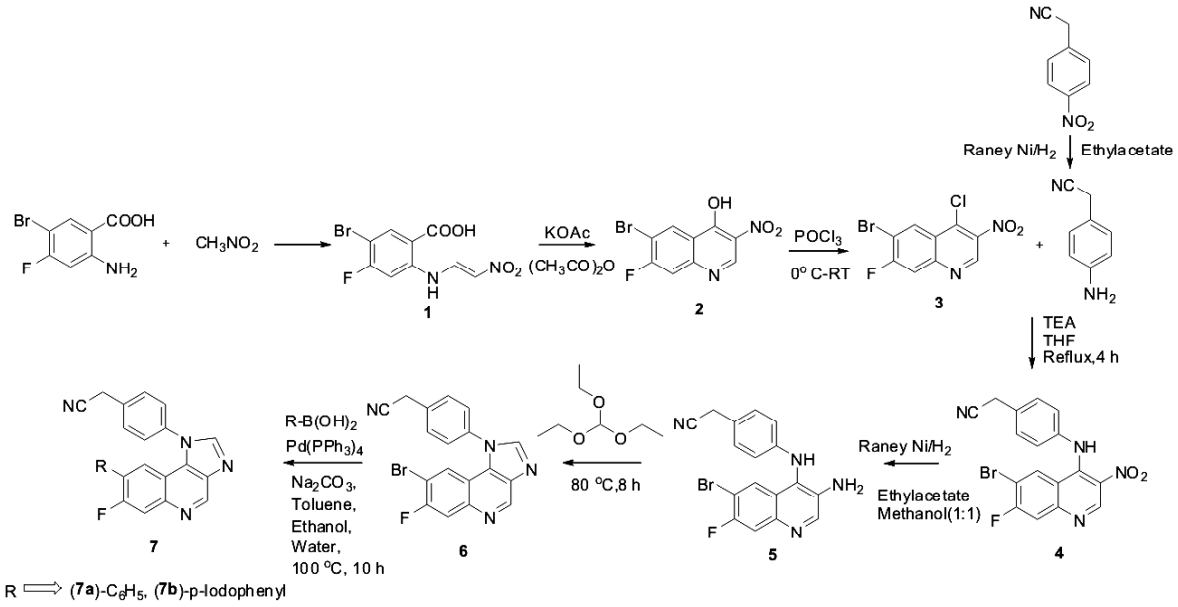

Fig. 1. Synthetic route for compounds **7a** and **7b**.

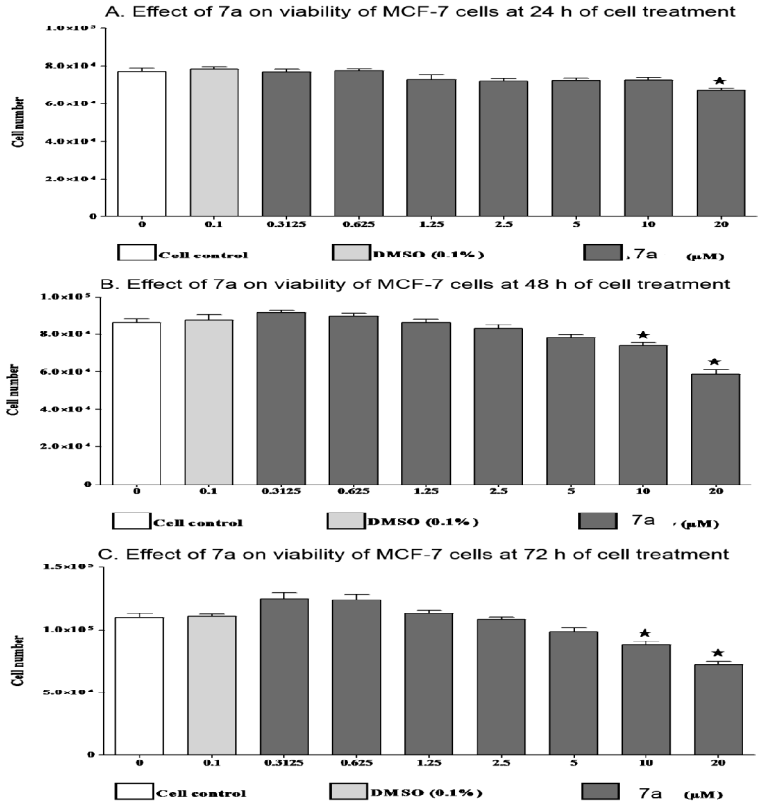

Fig. 2. Effect of **7a** on viability of MCF-7 cells at 24 h, 48 h and 72 h.

respectively (Fig. 2). Mitomycin-C was used as positive control and IC_{50} was found to be 15 μM at 48 h of treatment.

Molecular docking :

The docking study for compounds **7a** and **7b** was carried out using molecular docking server to predict the

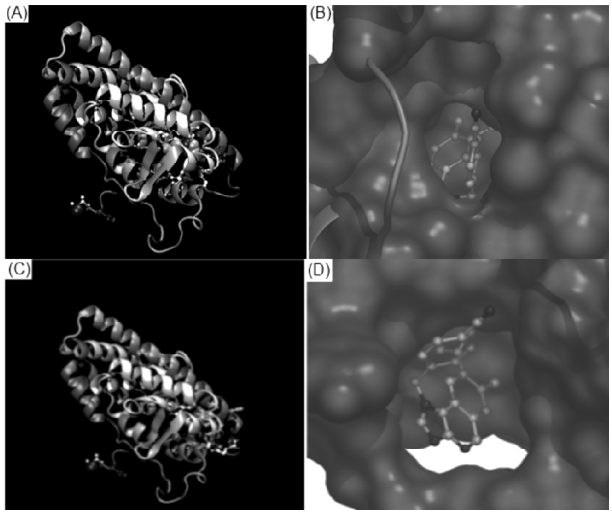

Fig. 3. (A) Binding mode of ligand **7a** in ribbon estrogen receptor, (B) Binding mode of ligand **7a** into cavity of estrogen receptor, (C) Binding mode of ligand **7b** in ribbon estrogen receptor and (D) Binding mode of ligand **7b** into cavity of estrogen receptor.

binding modes to the estrogen receptor (pdb : 3ERT). The docking study indicated a hydrogen bond in compound **7a** because of the interaction of nitrogen atom of cyano with oxygen atom of ASP351 (2.61 Å) with the binding energy –7.55 kcal/mol. Similarly in compound **7b** the hydrogen bond was observed due to the interaction of nitrogen atom of cyano with oxygen atom of TRP383 (3.03 Å) with the binding energy –8.23 kcal/mol. Binding mode of ligands with estrogen receptor is shown in Fig. 3. The shorter H-bond length (2.61 Å) of compound **7a** compared to **7b** (3.03 Å) indicate stronger interaction with estrogen receptor which in turn may be attributed to the higher proliferative activity of **7a** compared to **7b**.

Conclusion

New series of 1*H*-imidazo[4,5-*c*]quinoline derivatives were synthesized and characterized. Synthesised compounds 2-(4-(7-fluoro-8-phenyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile (**7a**) and 2-(4-(7-fluoro-8-(4-iodophenyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)acetonitrile (**7b**) have been screened for antiproliferative effect using MCF-7 (human breast cancer cell line). Among them, compound **7a** showed moderate inhibition compared to control.

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