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SYNTHESIS AND ANTICANCER EVALUATION OF NEW 1*H*-NAPHTHO[2,3-*D*] IMIDAZOLE-4,9-DIONE-1,2,3 TRIAZOLE HYBRIDS

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ABSTRACT

A new series of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-based 1,2,3-triazole hybrids (10a-10n) were synthesized by the reaction of 1-(prop-2-yn-1-yl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione with aromatic azides *via* copper-catalyzed azide-alkyne cycloaddition reactions. All the synthesized compounds are screened for their *in vitro* anticancer activities against three cancer cell lines namely MCF-7 (breast), HeLa (cervical), and A-549 (lung) using an MTT assay. The results revealed that the compounds 10a,10c,10g, and 10k exhibited promising activity against three cancer cell lines. Especially, compound 10a displayed higher activity against all the cell lines than the standard drug, The molecular docking studies of compounds 10a-10n as EGFR targeting agents were also supported by the observed IC_{50} data.

Keywords: 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione, *In vitro* Anticancer Activity, Molecular Docking, 1,2,3-Triazoles.

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INTRODUCTION

The quinone-building moiety, particularly naphthalene-1,4-dione, is an important biologically active pharmacophore in a number of chemotherapy agents, including Alkannin, Doxorubicin, and Mitoxantrone, as well as other biologically active natural products.[1] About a decade before, 2-morpholino ethyl amino-substituted naphthalene-1,4-dione [2] were rationally designed as inhibitors of the dual specificity protein phosphatase CDC25, which is a potential target for anticancer agents.[3–5] The CDC25 inhibitors mentioned above demonstrated potent anticancer activity *in vitro*. A 1-substituted imidazole part was recently added to the naphthoquinone framework, resulting in compounds with enhanced selectivity [6] while retaining high activity.[7–13] Encouraged by all these previous research, 1-substituted-1-(1,2,3-triazole)-1*H*-naphtho [2,3-*d*] imidazole-4,9-diones with an *N*-1,2,3-triazole moiety and a 1-substituted imidazole component on a naphthoquinone skeleton were developed to achieve high anticancer activity and selectivity. Copper-catalyzed azide-alkyne cycloaddition reaction has become one of the most significant reactions for the preparation of 1,2,3-triazoles.[14,15] In the last few decades, the importance of triazoles has gotten significant attention. Nowadays many studies have concentrated on the introduction of versatile biological, therapeutic, and pharmacological properties of 1,2,3 triazoles.[16,17] In the sight of the biological importance of 1*H*-naphtho[2,3-*d*]imidazole-4,9-diones and 1,2,3-triazoles, it was of considerable interest to develop new compounds incorporating both the ring systems. In this context, we decided to synthesize new derivatives of the 1,2,3 triazole-linked 1*H*-naphtho [2,3-*d*]imidazole-4,9-diones ring system *via* the copper-catalyzed click reactions.

EXPERIMENTAL

General Information

Physical properties (m. p) were recorded using uncorrected Stuart SMP10 digital equipment. A Shimadzu QP5050A quadrupole-based mass spectrometer was used to obtain Electrospray ionization mass spectra. Mass spectral data are given as m/z (Intensity). Bruker Ascend spectrometer was used for the recording of ^{1}H-NMR and ^{13}C-NMR spectra using $CDCl_3$ and DMSO-d_6 solvents. Chemical shifts are expressed in δ

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values (ppm) using the solvent peak as internal standard (TMS). All coupling constant (*J*) values are given in hertz. The abbreviations used are as follows s, singlet; d, doublet; m, multiplet. Thin layer chromatography monitored with silica gel precoated F254 Merck plates. All the solvents and reagents were commercially available and used without further purification. Synthesis of 2,3-Diamino-1,4-naphthoquinone (4a), 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (6a), 1-(Prop-2-yn-1-yl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (8a) was synthesised according to previous procedures.[18,19]

General Procedure for the Synthesis of 1-((1-phenyl-1*H*-1,2,3-triazol-4-yl) methyl)-1*H*-naphtho[2,3-*d*] imidazole-4,9-dione Derivatives (10a-10n)

To a 50 mL round bottom flask, 1-(prop-2-yn-1-yl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (8a) (1mmol), aryl azides (1.6 mmol) and CuI (0.1 mmol) in THF (10 mL) were added. Then the reaction mixture was allowed to stir under reflux at 60°C for 8 hours. The improvement of the reaction was checked by TLC. After completion of the reaction, the mixture was cooled to room temperature and extracted with ethyl acetate (2×15 mL). The crude products were obtained after the extracted organic layers were concentrated under a vacuum, dried over anhydrous Na_2SO_4, and washed with brine. Finally, the crude products were purified by column chromatography (60-120 silica gel) using (4:6) ethyl acetate-hexane as eluent to give desired pure products (10a-10n).

MTT Assay

A 96-well tissue culture microlitre plate was inoculated with 100L of complete medium containing 1×10^4 cells. Before the experiment, the plates were incubated for 18 hours at 37ºC in a humidified 5% CO_2 incubator. Following the removal of the medium, 100L of fresh medium containing the test compounds and doxorubicin at various concentrations such as 0.5, 1, and 2M was added to each well and incubated at 37ºC for 24 hours. The medium was then removed and replaced with 10L MTT dye. For 2 hours, the plates were incubated at 37ºC. The formed formazan crystals were dissolved in a 100L extraction buffer. A microplate reader was used to measure the optical density (O.D.) at 570 nm (Multi-mode Varioskan Instrument-Thermo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

Molecular Docking Studies

Auto dock 4.2 was used for molecular docking studies. The protein was downloaded from the protein data bank which is having PDB id 4HJO. Ligand and water are removed from the protein and calculated Gasteiger charges are after the addition of polar hydrogens. The ligands are drawn using chem 3D 14 and saved as a mol file after energy minimization. Then they are converted into PDB files using discovery studios. The grid box is generated by taking 60 points on three coordinate axes. Lamarckian GA (4.2) algorithm was employed to generate PDB files. Cygwin interface was used to obtain the dlg file from where the results were extracted. The 2D and 3D images are being rendered using Schrodinger's maestro v9.5 visualizer interface.

Characterization Data

1-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (8a)

M. p.: 196-198 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.33-8.24 (m, 1H), 8.18-8.07 (m, 2H), 7.82-7.68 (m, 2H), 5.32 (d, J=2.5 Hz, 2H), 2.66 (t, J=2.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 178.73, 176.80, 144.34, 143.31, 134.20, 133.62, 133.01, 132.81, 131.32, 127.05, 126.61, 76.53, 75.02, 36.93; MS (ESI):m/z = 237 $(M+H)^+$.

1-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10a)

M. p.: 273-275 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.32-8.21 (d, J = 7.1 Hz, 2H), 8.15 (s, 1H), 7.96 (s, 1H), 7.93 (d, J = 7.5 Hz, 2H), 7.86 -7.75 (m, 2H), 6.53(s, 1H) 5.42 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 179.41, 177.95, 145.82, 141.70, 139.54, 138.56, 138.31, 136.59, 135.21, 128.79, 128.51, 125.33, 125.02, 123.47, 45.81; MS (ESI):m/z=401 $(M+H)^+$.

1-((1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10b)

M. p.: 265-267 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.32 (d, J = 7.2, 1H), 8.23 (d, J = 7.2 Hz, 1H), 8.25(s, 1H), 8.01(s, 1H), 7.91 (d, J = 7.3 Hz, 2H), 7.77 – 7.65 (m, 4H), 5.65 (s, 2H).^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 179.98, 178.23, 144.82, 142.69, 138.56, 137.31, 136.59, 133.21, 128.79,

128.51, 127.85, 126.33, 125.02, 124.85, 123.47, 46.81; MS (ESI): m/z=424 $(M+H)^+$.

4-(4-((4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (10c)
M. p.: 253-255 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.37 (d, J = 7.1 Hz, 1H), 8.28 (d, J = 7.1 Hz, 1H), 8.15 (s, 1H), 7.92 – 7.83 (m, 4H), 7.75 (s, 1H), 7.66 (d, J = 7.5 Hz, 2H), 5.52 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 179.21, 177.95, 140.56, 140.38, 138.31, 136.59, 135.21, 131.79, 131.51, 127.33, 126.38, 125.22, 124.47, 121.12, 118.24, 44.81; MS (ESI):m/z = 381 $(M+H)^+$.

1-((1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10d)
M. p.: 244-246 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.31 (d, J = 7.2 Hz, 1H), 8.18 (d J = 7.2 Hz, 1H), 8.12 (s, 1H), 8.06 (s, 1H), 7.76-7.69 (m, 2H), 7.46 (m, 4H), 6.01(s, 1H), 5.41 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):178.86, 176.90, 155.87, 140.56, 139.32, 138.59, 137.42,136.29, 128.72, 128.51, 127.33, 125.41, 124.28, 118.36, 43.54; MS (ESI):m/z=372 $(M+H)^+$.

1-((1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10e)
M. p. :236-238 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.31 (d, J = 7.2 Hz, 1H), 8.25 (d, J = 7.2 Hz, 1H), 8.18 (s, 1H), 8.02 (s, 1H), 7.72 (d, J = 7.4 Hz, 2H), 7.66-7.58 (m, 2H), 7.19 (d, J = 7.8 Hz, 2H), 5.52 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):179.42, 177.82, 159.22, 158.61, 140.56, 140.32, 138.59, 136.21, 135.23, 134.20, 129.94, 129.52, 127.31, 125.87, 125.62, 124.71, 119.34, 118.03, 45.05; MS (ESI):m/z = 374 $(M+H)^+$.

1-((1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10f)
M. p. :246-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.27 (d, J = 7.1 Hz, 1H), 8.21 (d, J = 7.1 Hz, 1H), 8.15 (s, 1H), 8.04 (s, 1H), 7.67 (d, J = 7.2 Hz, 2H), 7.61-7.55 (m, 2H), 7.16 (d, J = 7.5 Hz, 2H), 5.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):178.72, 176.81, 156.18, 154.32, 141.52, 139.61, 137.51, 136.42, 135.01, 133.29, 128.91, 128.55, 127.32, 125.86, 125.45, 124.24, 119.31, 117.03, 45.01; MS (ESI):m/z = 390 $(M+H)^+$.

1-((1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10g)
M. p. :279-281 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.27 (d, J = 7.1 Hz, 1H), 8.21 (d, J = 7.1 Hz, 1H), 8.15 (s, 1H), 8.04 (s, 1H), 7.67 (d, J = 7.2 Hz, 2H), 7.61-7.55 (m, 2H), 7.16 (d, J = 7.5 Hz, 2H), 5.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):178.56, 176.21, 152.18, 150.28, 140.57, 138.64, 137.56, 136.47, 134.89, 133.25, 128.25, 127.51, 127.21, 125.41, 125.06, 123.21, 118.32, 117.52, 44.81; MS (ESI):m/z = 434 $(M+H)^+$.

1-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10h)
M. p.: 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.31 – 8.28 (m, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.15 (s, 1H), 8.01 (s, 1H), 7.92-7.87 (m, 2H), 7.65 (d J = 7.5 Hz, 2H), 7.36 (m, 2H), 7.18-7.11 (m, 1H), 5.56 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):181.41, 178.91, 141.56, 139.87, 138.28, 136.05, 135.28, 130.71, 129.57, 128.11, 127.46, 125.33, 124.29, 122.38, 46.81; MS (ESI):m/z=356 $(M+H)^+$.

1-((1-(p-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10i)
M. p.: 234-236 °C;^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.25 (d, J = 7.2 Hz, 1H), 8.22 (d J = 7.2 Hz, 1H), 8.11 (s, 1H), 7.92 (s, 1H), 7.79-7.68 (m, 2H), 7.4 (d, J = 7.5 Hz, 2H), 7.3 (d, J = 7.1 Hz, 2H), 5.49 (s, 2H), 2.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):180.49, 177.91, 140.56, 139.35, 138.81, 136.56, 135.42, 133.29, 128.78, 127.51, 127.04, 125.33, 123.47, 123.16, 45.12, 25.39; MS (ESI):m/z=370 $(M+H)^+$.

1-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10j)
M. p.: 239-241 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.39 (d, J = 6.5 Hz, 1H), 8.31 (d, J = 7.0 Hz, 1H), 8.26 (s, 1H), 8.19 (s, 1H), 7.96-7.87 (m, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.24 (d, J = 7.4 Hz, 2H), 5.52 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 181.29, 179.42, 156.28, 140.51, 140.29, 138.59, 133.25, 130.18, 128.71, 127.28, 127.01, 125.47, 124.80, 118.69, 61.15, 48.64; MS (ESI) : MS (ESI) : m/z=396 $(M+H)^+$.

1-((1-(4-isopropylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10k)
M. p.: 247-249 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.25 (d, J = 7.2 Hz, 1H), 8.24 (d, J = 7.2 Hz,

1H), 8.19 (s, 1H), 8.04 (s, 1H), 7.82 (m, 2H), 7.35 (d, *J* = 7.5 Hz, 2H), 7.01 (d, *J* = 7.5 Hz, 2H), 5.41 (s, 2H), 2.85 (dt, *J* = 12.1, 6.4 Hz, 1H), 1.52 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 178.74, 176.28, 143.85, 140.68, 140.31, 139.16, 136.27, 135.85, 131.79, 131.16, 127.29, 127.29, 125.31, 123.19, 44.12, 32.15, 25.26; MS (ESI) : MS (ESI) : m/z=398 $(M+H)^+$.

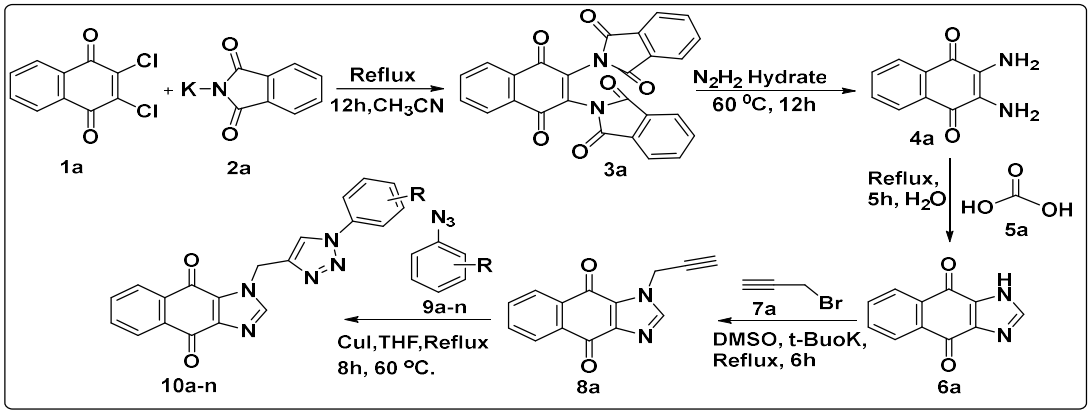
1-((1-(pyridin-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10l)
M. p.: 238-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.52 (d, *J* = 3.0 Hz, 1H), 8.31 (d, *J* = 3.0 Hz, 1H), 8.26 (d, *J* = 3.0 Hz, 1H), 8.17 (s, 1H), 8.01 (m, 2H), 7.95 (s, 1H), 7.91-7.84 (m, 2H), 7.41 (t, *J* = 7.3 Hz, 1H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm):180.01, 177.23, 151.85, 143.25, 141.56, 140.31, 137.29, 135.59, 134.28, 133.41, 130.28, 130.29, 127.37, 126.29, 125.41, 46.75; MS (ESI) : m/z=357 $(M+H)^+$.

1-((1-(furan-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10m)
M. p.: 220-222 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.32 (d, *J* = 7.2 Hz, 1H), 8.26 (d, *J* = 7.2 Hz, 1H), 8.18 (s, 1H), 8.09 (s, 1H), 7.88-7.80 (m, 2H), 6.95 (dd, *J* = 7.1, 1.6 Hz, 1H), 6.68 (d, *J* = 7.1, Hz, 1H), 6.27 – 6.22 (d, *J* = 7.1 Hz 1H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 180.25, 178.29, 142.49, 140.52, 139.51, 138.31, 136.35, 132.69, 130.58, 129.78, 127.79, 126.47, 110.91, 105.29, 47.27; MS (ESI) : MS (ESI) : m/z=346 $(M+H)^+$.

1-((1-(thiophen-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10n)
M. p.: 235-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 8.27 (d, *J* = 7.1 Hz, 1H), 8.23 (d, *J* = 7.1 Hz, 1H), 8.19 (s, 1H), 7.95 (s, 1H), 7.78-7.69 (m, 2H), 7.20-7.12 (d, J=7.2 Hz, 1H), 7.01-6.95 (d, J = 7.0Hz, 1H), 6.83 (m, 1H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ in ppm): 178.35, 176.42, 141.89, 138.52, 137.59, 136.42, 135.89, 132.61, 130.71, 128.56, 127.58, 125.19, 120.43, 116.71, 44.62; MS (ESI) : MS (ESI) : m/z = 362 $(M+H)^+$.

RESULTS AND DISCUSSION

The synthetic approach to desired regioselective 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,3-triazole hybrids (10a-10n) was presented in Scheme-1. In the first, the 2,3-diaminonaphthalene-1,4-dione (4a) is synthesized by Gabriel reaction between 2,3-dichloronaphthalene-1,4-dione (1a) and potassium phthalimide (2b).[20] The intermediate 2,3-diaminonaphthalene-1,4-dione reacted with carbonic acid in water under reflux conditions for 5h to afford the corresponding 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (6a). The *N*-alkylation on 1*H*-naphtho[2,3-*d*] imidazole-4,9-dione with propargyl bromide using *t*-BuOK as a base in DMSO solvent under reflux for 6 hours produces 1-(prop-2-yn-1-yl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (8a). Finally, the desired regioselective 1*H*-naphtho[2,3-*d*] imidazole -4,9-dione-1,2,3-triazole hybrids were produced by the 1,3-dipolar cycloaddition reaction between aryl azides (9a-9n) and (8a) terminal alkynes in the presence of CuI in THF solvent at 60 ^{0}C for 8 hours (10a-10n). The yields are presented in Table-1.



Scheme-1: Synthesis of 1,2,3 triazole-1*H*-naphtho[2,3-*d*] imidazole-4,9-dione Hybrids

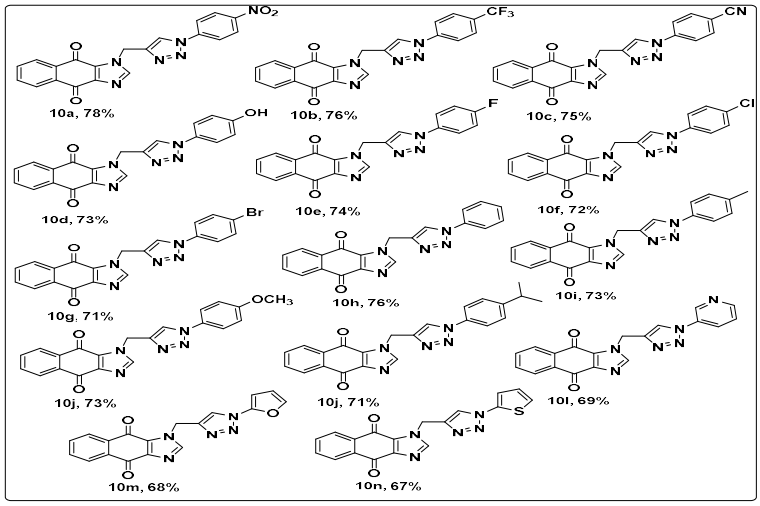


Table-1: Substrate Scope

In-vitro Anticancer Studies

The newly synthesized hybrid molecules (10a-10n) were studied for their *in vitro* anticancer contrary to three human cancer cells lines such as MCF-7 (breast), HeLa (cervical), and A-549 (lung) by using MTT[21] assay and obtained results were represented in Table-2. Assay and outcomes revealed that compound 10a showed higher potency against three cell lines than the standard drug Doxorubicin, while three compounds 10c, 10g, and 10k displayed the most promising potency against all cell lines as compared to positive control. The structure-activity relationship (SARs) of these compounds showed that compound 10a with the 4-nitro group on phenyl moiety linked to the 1,2,3-triazole ring displayed greater activity among all the cell lines than the standard drug. Compound 10g with a 4-bromo substituent on phenyl moiety attached to the 1,2,3-triazole ring has shown greater potency against MCF-7 and A549 as compared to standard drugs. Besides, compounds 10c and 10k containing 4-bromo and 4-isopropyl substituent on phenyl moiety respectively showed the most promising potency against all cell lines as related to standard drugs. Introductions of heterocyclic moieties on the phenyl ring resulting in compounds 10l, 10m, and 10n were shown less potent as related to the standard doxorubicin. All the remaining compounds exhibited good to moderate activity against the standard drug. Exclusively, the efficacy order of potent compounds against MCF-7 is as follows: 10a>10g>10k>10c.

Table-2: In Vitro Anticancer Activity of Newly Synthesized Compounds (10a-10n) with IC_{50} in μM [(a)]

Entry	R	[a]MCF-7	[b]Hela	[c] A-549
10a	-4 NO_2	1.53 ± 0.23	4.52±0.65	1.45 ± 0.25
10b	-4 CF_3	5.04±0.26	9.26±0.52	6.45±0.33
10c	-4 CN	3.34±0.56	7.05±0.91	5.74±0.82
10d	-4 OH	5.51±0.52	8.92±0.65	6.12±0.25
10e	-4 F	6.62 ± 0.77	-	7.52 ± 0.42
10f	-4 Cl	7.12±0.95	8.53 ±1.22	-
10g	-4 Br	1.86 ± 0.25	6.55±.35	1.75 ± 0.12
10h	- H	7.12±1.21	-	8.85±1.21
10i	- 4 CH_3	6.86±0.35	9.10 ± 0.61	7.72 ± 0.25
10j	- 4 OCH_3	8.25 ± 0.19	9.96±0.20	6.54 ± 0.25
10k	- 4 Isp	2.85 ± 0.25	5.95±0.25	3.58 ± 0.15
10l	-Pyridyl	7.25±0.26	9.92 ± 0.54	8.62 ± 0.21
10m	-C_4H_4O	8.22 ± 0.28	9.18±0.29	-
10n	- C_4H_4S	10.87 ± 0.36	-	9.78 ± 0.29
Doxorubicin		2.18±0.18	5.51±0.039	2.02±0.17

ND=Not determine. [(a).] Each data represents mean ± S.D values. Three different experiments were performed in triplicates.

Molecular Docking Studies

Tyrosine Kinase EGFR Inhibitory Activity

One of the main targets for developing anti-cancer drugs was the EGFR.[22] This protein acts as a cell-surface receptor and is essential for the mammary glands' ductal development.[23] Many types of cancer are caused by the overexpression of the EGFR.[24-26] Previously Naphthoquinone and Imidazo based 1,2,3 triazoles as EGFR-targeting anticancer agents for also studied.[27] In view of this, various 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,3-triazoles compounds were synthesized as EGFR inhibitors. The molecular docking study of the synthesized compounds 10a-10n was studied in the binding site of the anticancer EGFR protein (PDB ID: 4JHO) binding domain using the AUTODOCK 4.2 version and the Fig's are ablatively reduced using Schrodinger's maestro v9.5 visualizer boundary docking values for anticancer studies. The active compounds (10a-10n) from anticancer studies are further supported by docking studies through molecular interactions. Molecular docking studies revealed that compounds 10a, 10c, 10g, and 10k showed better docking scores than the remaining molecules. Specifically, compound 10a displayed the best docking score of -11.86. Thus, the 2D and 3D interaction diagram of the ligand 10a with the complex protein is shown in Fig.-1 and 2.

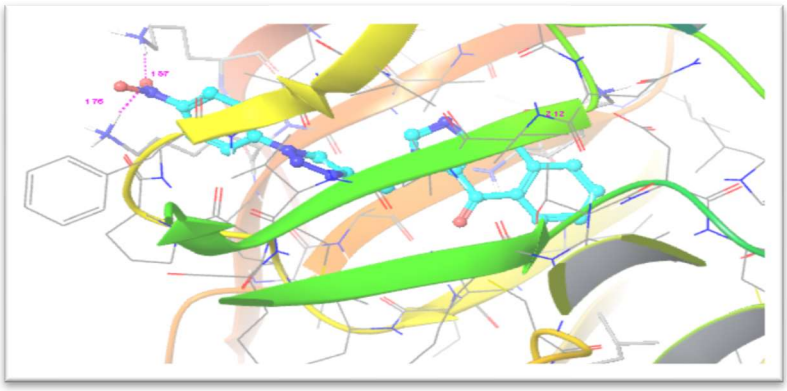


Fig.-2: 3D Interaction of Compound 10a with EGFR

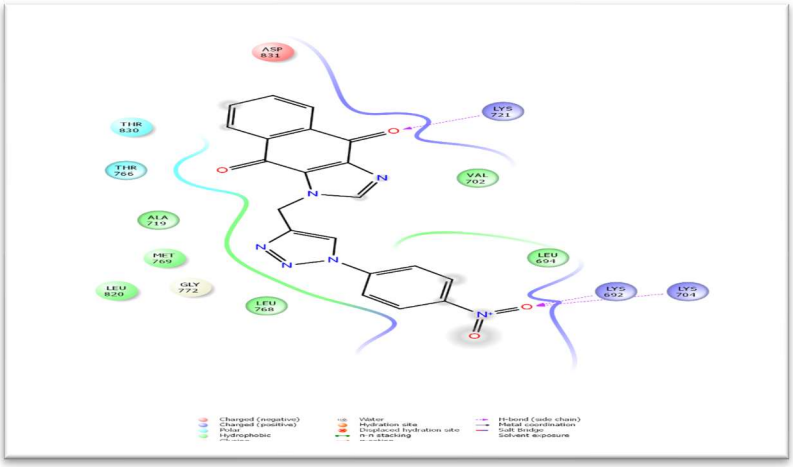


Fig.-2: 2D Interaction of Compound 10a with EGFR

Table-3: Molecular Docking Values.

S. No.	Compd	Binding interaction Energy (kcal/mol)	Inhibition Constant (nM)	No. of hydrogen bonds	Residues involved in hydrogen bonding	Run
1.	10a	-11.86	2.02	3	LYS721, LYS704, LYS692	6
2.	10b	-9.69	79.58	0	0	8

3.	10c	-10.94	9.64	1	LYS721, MET769	9
4.	10d	-9.08	221.53	1	ALA847, TYR845	6
5.	10e	-10.09	40.04	2	LYS721, ASP831	2
6.	10f	-10.38	24.51	1	LYS721	3
7.	10g	-11.07	7.67	1	MET 769	7
8.	10h	-10.25	30.92	1	MET 769	1
9.	10i	-10.30	28.03	1	MET 769	3
10.	10j	-9.98	48.29	1	LYS721	6
11.	10k	-11.10	7.35	1	MET 769	9
12.	10l	-10.10	39.34	1	LYS 721	2
13.	10m	-9.75	70.92	2	LYS721, PHE 831	5
14.	10n	-10.51	19.77	1	LYS 721	6

CONCLUSION

Here, we have described the synthesis of some new 1,2,3 triazole–1*H*-naphtho[2,3-*d*]imidazole-4,9-dione hybrids (10a-10n) using Gabriel and 1,3-dipolar cycloaddition reactions. Among the derivatives, compound 10a has exhibited superior activity against all the cell lines than the standard medication, doxorubicin. Also, when compared to doxorubicin, the compounds 10c, 10g, and 10k displayed the most promising activity. Outcomes of molecular docking studies of compounds 10a-10n as EGFR targeting agents were also found to be consistent with the experimental IC_{50} data.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. J. Zhang, N. Redman, A. P. Litke, J. Zeng, J. Zhan, K. Y. Chan, and C. W. T. Chang, *Bioorganic & Medicinal Chemistry*, **19(1),** 498 (2011), https://doi.org/10.1016/j.bmc.2010.11.001
2. T. S. Hsu, C. Chen, P. T. Lee, S. J. Chiu, H.F. Liu, C.C. Tsai, and J.I. Chao, *Cancer Chemotherapy and Pharmacology*, **62(5),** 799(2008), https://doi.10.1007/s00280-007-0667-5
3. S. Keinan, W. D. Paquette, J. J. Skoko, D. N. Beratan, W. Yang, S. Shinde, P. A. Johnston, J. S. Lazo, and P. Wipf, *Organic & Biomolecular Chemistry*, **6(18)**, 3256(2008), https://doi.org/10.1039/B806712K
4. J. Cossy, D. Belotti, M. Brisson, J. J. Skoko, P. Wipf, and J. S. Lazo, *Bioorganic & Medicinal Chemistry*, **14(18)**, 6283(2006), https://doi.org/10.1016/j.bmc.2006.05.053
5. Zhanxiong Liu, Zhenfeng Zhang, Wanbin Zhang, Deyue Yan, *Bioorganic & Medicinal Chemistry Letters,* **28**, 2454(2018), https://doi.org/10.1016/j.bmcl.2018.06.007
6. Z. Liu, J. Yuan, Z. Zhang, D. Yan, W. Zhang, *Chinese Journal of Organic Chemistry,* **38(12)**, 3302 (2018), https://doi.org/10.6023/cjoc201807019

7. S.H.S. Ho, M.Y. Sim, W.L.S. Yee, T. Yang, S.P.J. Yuen, and M. L. Go, *European Journal of Medicinal Chemistry*, **104,** 42 (2015), https://doi.org/10.1016/j.ejmech.2015.09.026
8. J.S. Kim, H. J. Lee, M. E. Suh, H. Y. P. Choo, S. K. Lee, H. J. Park, C. Kim, S.W. Park, and C.O. Lee, *Bioorganic & Medicinal Chemistry*, **12(13)**, 3683(2004), https://doi.org/10.1016/j.bmc.2004.04.014
9. H.W. Yoo, M. E. Suh, S. W. Park, *Journal of Medicinal Chemistry*, **41(24)**, 4716(1998), https://doi.org/10.1021/jm970695n
10. Z. Liu , Z. Zhang , W. Zhang , D. Yan, *Bioorganic & Medicinal Chemistry Letters*, **28(14)**, 2454 (2018), https://doi.org/10.1016/j.bmcl.2018.06.007
11. S. Chu Kuo, T. Ibuka, L. Jiau Huang, J. Cherng Lien, S. Ren Yean, S. Chieh Huang, D. Lednicer, S. M. Natschke, K. Hsiung Lee, *Journal of Medicinal Chemistry,* **39 (7)**,1447(1996), https://doi.org/10.1021/jm950247k
12. D. Choudhari, S.G. Sunita, D.Chakravarty, S.R. Shaikh, D. N. Lande, P. G. Shridhar, Pradeep Kumar Rao, S.Surekha, V. G. Puranik, G.Rajesh, *New Journal of Chemistry***, 44,** 6889(2020), https://doi.org/10.1039/C9NJ04339J
13. L. M. Gornostaev, M. V. Vigant, O.I. Kargina, *Russian Journal of Organic Chemistry*, **49**, 1354(2013), https://doi.org/10.1134/S1070428013090194
14. D. Gouthamia, B. Srinivasa, and M. Ravindera, *Russian Journal of Bioorganic Chemistry*, **47 (4)**, 882(2021), https://doi.org/10.1134/S1068162021040105
15. V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angewandte Chemie,* **114(14),** 2708 (2002),https://doi.org/10.1002/1521-3757(20020715)114:14<2708::AID-ANGE2708>3.0.CO;2-0
16. B. Tozkoparan, E. Kupeli, E. Yesilada, M. Ertan, *Bioorganic & Medicinal Chemistry,* **15(4)**, 1808 (2007), https://doi.org/10.1016/j.bmc.2006.11.029
17. S.R. Patpi, L. Pulipati, P. Yogeeswari, D. Sriram, N. Jain, B. Sridhar, R. Murthy, D.T. Anjana, S.V. Kalivendi, S. Kantevar, *Journal of Medicinal Chemistry,* **55(8)**, 3911(2012), https://doi.org/10.1021/jm300125e
18. M. Devi, P. Kumar, R.Singh, J.Sindhu, K.Ramesh, *European Journal of Medicinal Chemistry,* **250 (15),**115230 (2023), https://doi.org/10.1016/j.ejmech.2023.115230
19. D.Choudhari, S.G. Sunita, D. Chakravarty, D. N. Lande,P. G. Shridhar , Pradeep Kumar Rao, S. Surekha, V. G. Puranik, G. Rajesh, *New Journal of Chemistry*, **44**, 6889(2020), https://doi.org/10.1039/C9NJ04339J
20. J. McBryan, J. Howlin, S. Napoletano, F. Martin, *Journal of Mammary Gland Biology and Neoplasia*, **13,** 159 (2008), https://doi.org/10.1007/s10911-008-9075-7
21. G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba, A. A. Genazzani, *Medicinal Research Reviews*, **28(2)**, 278 (2008), https://doi.org/10.1002/med.20107
22. R. Roskoski Jr, Pharmacological Research, **87,** 42(2014),https://doi.org/ 10.1016/j. phrs. 2014.06.001
23. J. Sebastian, R. G. Richards, M. P. Walker, J. F. Wiesen, Z. Werb, R. Derynck, Y. K. Hom, G. R. Cunha, R. P. DiAugustine, *Cell Growth and Differentiation*, **9**, 777(1998)
24. F. Walker, L. Abramowitz, D. Benabderrahmane, X. Duval, V. Descatoire, D. Henin, T.Lehy, T. Aparicio*, Human. Pathalogy,* **40,** 1517(2009), https://doi.org/10.1016/j.humpath.2009.05.010
25. T. Liang, X. Sun, W. Li, G. Hou, F. Gao, *Frontiers in Pharmacology,* 1374, *(*2021), https://doi.org/10.3389/ fphar.2021.661173
26. P. Mahalapbutr, R. Leechaisit, A. Thongnum, D. Todsaporn, V. Prachayasittikul, T. Rungrotmongkol, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, R. Pingaew, *ACS omega***, 7(21)**, 17881(2022), https://doi.org/10.1021/acsomega.2c01188
27. R. Guda, G. Kumar, R. Korra, S. Balaji, G. Dayakar, R. Palabindela, P. Myadaraveni, N. R. Yellu, M. Kasula. *Journal of Photochemistry and Photobiology B: Biology,* **176**, 69(2017), https://doi.org/10.1016/j.jphotobiol.2017.09.010
28. G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba, A. A. Genazzani, *Medicinal Research Reviews,* **28(2**), 278(2008), https://doi.org/10.1002/med.20107

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