2023
10(1), 506

Chemical Biology
LETTERS

Synthesis, characterization and anticancer evaluation of new 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,4-oxadiazole hybrids

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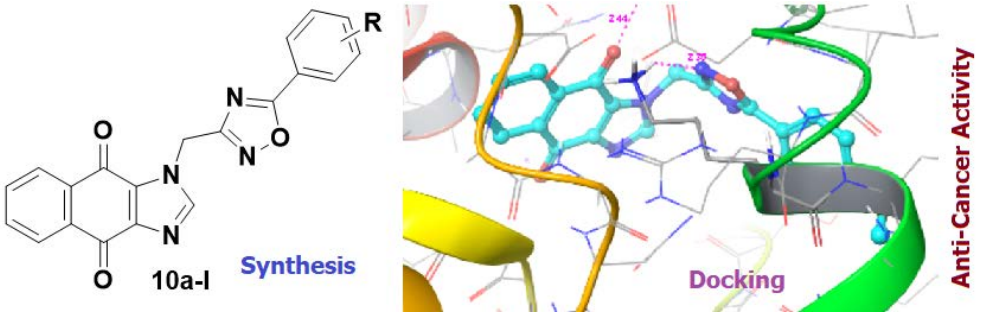
Submitted on: 17-Nov-2022, Accepted and Published on: 10-Jan-2023

Article

ABSTRACT

New series of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,4 oxadiazoles (**10a-10l**) synthesized using $NH_2OH.HCl/Et_3N$ and $POCl_3$/DMF (Vilsmeier reagent) mediated one-pot reaction between 2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)acetonitrile and several aromatic carboxylic acids as key approach have been reported here. All synthesized compounds were screened for the *in vitro* cytotoxicity against three human cancer cell lines such as A549, PC3, and MCF-7. Three compounds (**10d**, **10f** and **10k**) exhibited superior activity than the standard etoposide against all the cell lines with IC_{50} values <2 µM. Finally, molecular docking studies revealed the important binding interactions of potent compounds **10d**, **10f** and **10k** with the α, β-tubulin (PDB ID-1SA0).



Keywords: 1H-naphtho[2,3-d]imidazole-4,9- dione , In vitro anticancer activity, Molecular docking, 1,2,4-Oxadiazole

INTRODUCTION

Cancer is a leading cause of health problems with a continuous development in large number of patients throughout the world.[1] The World Health Organization (WHO) was anticipated that the human deaths as a results of cancer will reach to around 12 million till the year 2030. Although, we have huge development in the discovery of anticancer drugs, few limitations such as low selectivity, less efficiency and possible drug resistance have always motivated the medicinal chemists to develop new anticancer drugs.

It is noteworthy that the 1,2,4-oxadiazole establishes bioisosteric equality with amide and acid moieties, owing to the probability of specific interaction (e.g., hydrogen bonding),[2] and thus extensively producing several analogues with diverse biological activities.[3,4] Currently, few drugs like Ataluren, Butalamine, Fasiplon, Prenoxdiazine, Oxolamine, Pleconaril and Proxazole present in market are containing 1,2,4-oxadiazole nucleus as basic core.[5–7] Predominantly, among all oxadiazole isomers, the 1,2,4-oxadiazole is mostly present in natural products. For instance, naturally available Phidianidine A and Phidianidine B showed potent *in vitro* cytotoxic activity against various tumour and non-tumour mammalian cell lines.[8,9] The stimulating revolution came with the discovery of 3,5-diaryl-1,2,4-oxadiazoles as apoptosis inducers,[10] plentiful 1,2,4-oxadiazole compounds were then synthesized as potent anticancer agents.[11–13]

On the other aspect, the quinone structural motif, particularly naphthoquinone, is a very important framework in several chemotherapy agents, such as Doxorubicin, Alkannin and Mitoxantrone and other biologically active natural products.[14,15] About a decade ago, 2-morpholino ethylamino-substituted naphthoquinones[16] were designed rationally as inhibitors for the dual specificity protein phosphatase CDC25, that considered to be a potential targets for the development of anticancer agents.[17–19] Recently, 1-substituted imidazole segment has been appended to the naphthoquinone framework and the resulting compounds have also shown increased selectivity with retaining high activity.[20–22]

Based on all the above facts and based on necessity to develop potent, safe and selective anticancer agents in the current medicinal chemistry research with hybrid molecules,[23,24] in the present work, we, designed and synthesized some new 1,2,4 oxadiazole-linked 1*H*-naphtho [2,3-d]imidazole-4,9-dione derivatives as *in vitro* cytotoxic agents.

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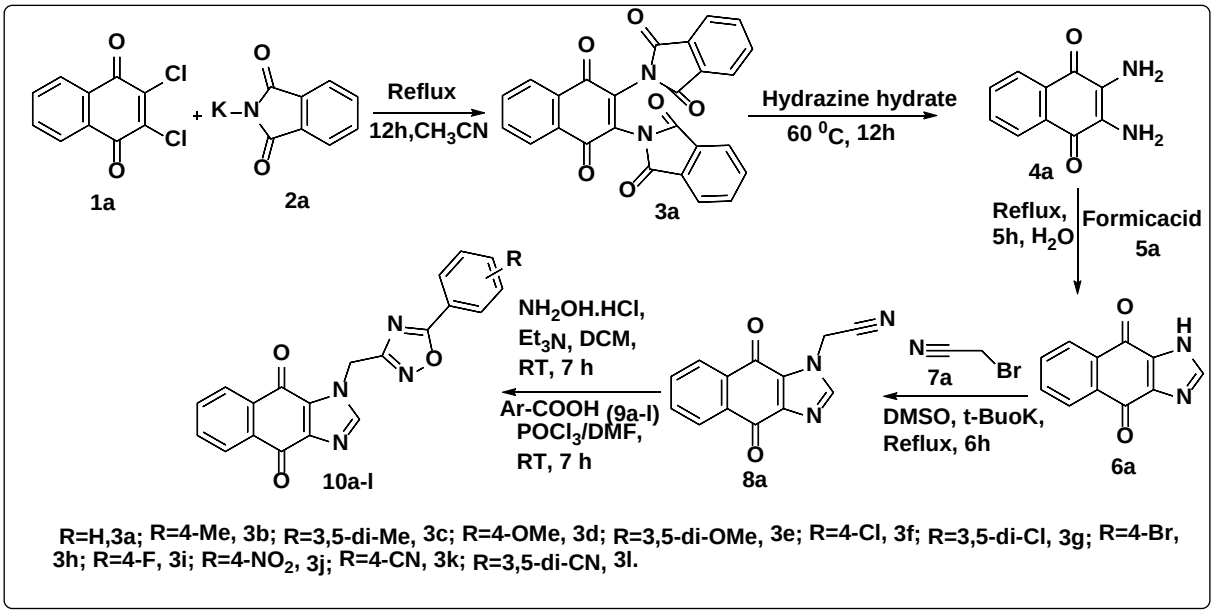
URN:NBN:sciencein.cbl.2023.v10.506
 ISSN: 2347–9825
https://pubs.thesciencein.org/cbl

RESULTS AND DISCUSSION

Chemistry

The synthetic approach to desired 1*H*-naphtho[2,3-*d*] imidazole-4,9-dione-1,2,4-oxadiazole hybrids **(10a-10l)** was presented in **scheme 1**. At first, the diamino-1,4-naphthoquinone (**4a**) was synthesized from 2,3-dichloro-1,4-naphthoquinone (**1a**) by Gabriel synthesis using potassium phthalimide (**2a**) followed by hydrazine hydrate.[25] A one-pot procedure was then used for the conversion of diamino-1,4-naphthoquinone (**4a**) into 1*H*-naphtho[2,3-*d*] imidazole-4,9-dione (**6a**).[25] Later, 2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)acetonitrile (**8a**) was synthesized from reaction of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (**6a**) with 2-bromoacetonitrile (**7a**) using ^{t}BuOK in DMSO at 60 ^{0}C after 6 hours. Finally, we, utilized the synthetic application of previously reported one-pot method of disubstituted 1,2,4-oxadiazoles, in order to fulfil few green chemistry principles, like synthetic efficiency, operational simplicity, atom economy and energy consumption etc.[26] In aspect, the intermediate (**8a**) was reacted with $NH_2OH.HCl$ and Et_3N in dry DCM at RT 7 hours to afford corresponding *in situ* (*E*)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)-N'-hydroxyacetimidamide**,** which was consequently reacted with few aromatic carboxylic acids (**9a-9l**) by means of Vilsmeier reagent at same temperature for further 7 hours to afford final compounds (**10a-10l**) in moderate to good yields.



Scheme 1. Synthesis of 1,2,4oxadiazole–1*H*-naphtho[2,3-*d*]imidazole-4,9- dione hybrids

In vitro cytotoxicity

All the newly synthesized compounds (**10a–10l**) were further investigated for their *in vitro* cytotoxicity against three human cancer cell lines like breast (MCF-7), prostate (PC3) and lung (A549) using MTT assay. The etoposide used as standard drug and results are conveyed in IC_{50} with μM. The results of **table 1**, revealed that the compound **10d, 10f**, and **10k** displayed superior activity against all the cell lines than the standard drug. Predominantly, three compounds such as **10e**, **10g** and **10l** showed most promising potency against all cell lines when compared with positive control.

The effect on the *in vitro* cytotoxicity by nature of substituent on the phenyl ring attached to 1,2,4-oxadiazol core moiety was also analyzed using structure-activity relationship (SAR) studies. In the case of electron-donating substituents, compound **10d** containing 4-methoxy substituent displayed more potency. In this manner, introducing more methoxy substituents on phenyl ring led to compounds **10e** which had lesser potency than the compound **10d**. However, compound **10a** containing simple phenyl ring **(10a)** or (**10b** and **10c**) containing methyl substituents were less potent than the methoxy compounds **10d** and **10e**.

In the case of electron withdrawing substituents, the compound **10f** containing 4-Cl substituent showed higher activity. The next better activity was displayed by the compound **10k** which had 4-CN substituent. However, compounds **10g** and **10l** having 3,5-diCl and 3,5-diCN substituents respectively had lesser activity as compared to compounds **10f** and **10k**. On the other aspect, compounds **10h**, **10i** and **10j** containing 4-Br, 4-Cl and 4-NO_2 substituents respectively were very less potent than the compounds **10f**, **10g**, **10k** and **10l**.

Molecular docking studies:

The molecular docking studies for the potent compounds **10d**, **10f** and **10k** were carried out by taking α, β-tubulin (PDB ID-1SA0) as the target protein. The results of **table 2** revealed that the compound **10d** exhibited highest binding energy (-9.64 kcal/mol) and inhibition constant (13.47 micromolar) with the protein. It formed hydrogen bond with ASN249 and LYS254 residues having bond length 2.35 Å and 2.44 Å.

Table 1. In vitro anticancer activity of newly synthesized compounds with IC_{50} in µM.

Entry	R	[a]MCF-7	[b]PC3	[c]A549
10a	H	6.25±0.39	ND	8.59±1.14
10b	4-CH_3	5.12±0.31	5.59±1.89	8.28±1.18
10c	3,5-diCH_3	9.25±0.89	10.01±0.9	7.23±0.89
10d	4-OCH_3	0.082± 0.01	0.91±0.65	0.96±0.082
10e	3,5-diOCH_3	2.51±0.035	2.49±0.05	3.01±0.035
10f	4-Cl	0.094±0.19	0.98±0.24	0.96±0.062
10g	3,5-diCl	3.32±0.068	4.98±2.33	5.09±0.28
10h	4-Br	6.22±0.58	ND	7.02±1.11
10i	4-F	7.12±0.74	ND	ND
10j	4-NO_2	6.10±0.49	7.05±3.03	ND
10k	4-CN	1.062±0.010	1.73±0.54	1.98±0.067
10l	3,5-diCN	3.52±0.009	3.92±0.61	4.79±0.059
Etoposide		2.11±0.024	2.39±1.56	3.08±0.135

ND=Not determine. Each data represents as mean ± S.D values. From three different experiments performed in triplicates; a) MCF-7: breast cancer cell line; b) PC3: human prostate cancer cell line; c) A549: lung cancer cell line;

Next, compound **10f** exhibited almost similar binding energy -8.49 kcal/mol inhibition constant 17.40 micromolar with zero hydrogen bond. Similarly, compound **10k** have exhibited nearly comparable binding energy -7.79 kcal/mol and inhibition constant 306.34 in micromolar. The compound **10k** has formed two hydrogen bonds with ARG123 residue having bond lengths 2.24 Å, and one hydrogen bond with TYR161 residue having bond length 2.78 Å. Thus, the 2D and 3D interaction diagram of the ligand **10d** with the complex protein is shown in **figure 1**.

Table 2: Molecular docking results:

Comp.	Binding Energy (kcal/mol)	Inhibition Constant (uM)	No. of hydrogen bonds	Residues involved in hydrogen bonding	Run
10d	-9.64	13.47	2	ASN249, LYS254	5
10f	-8.49	17.40	0	0	6
10k	-7.79	306.34	2	ARG123, TYR161	10

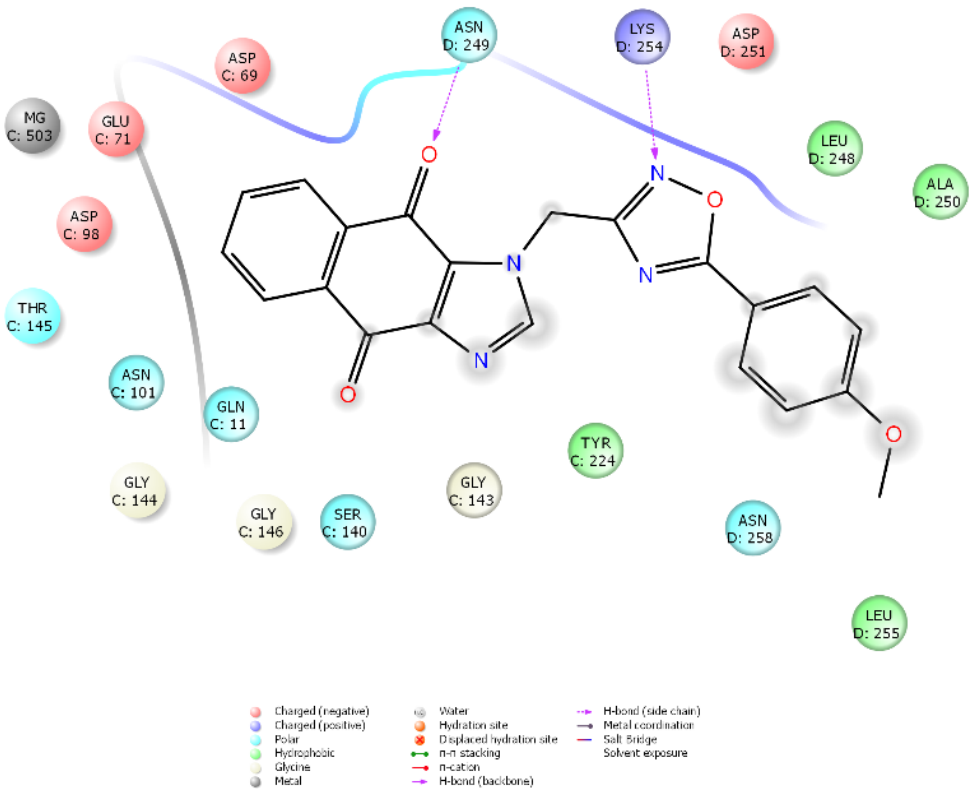


Figure 1: 2D interaction of compound 10d with EGFR

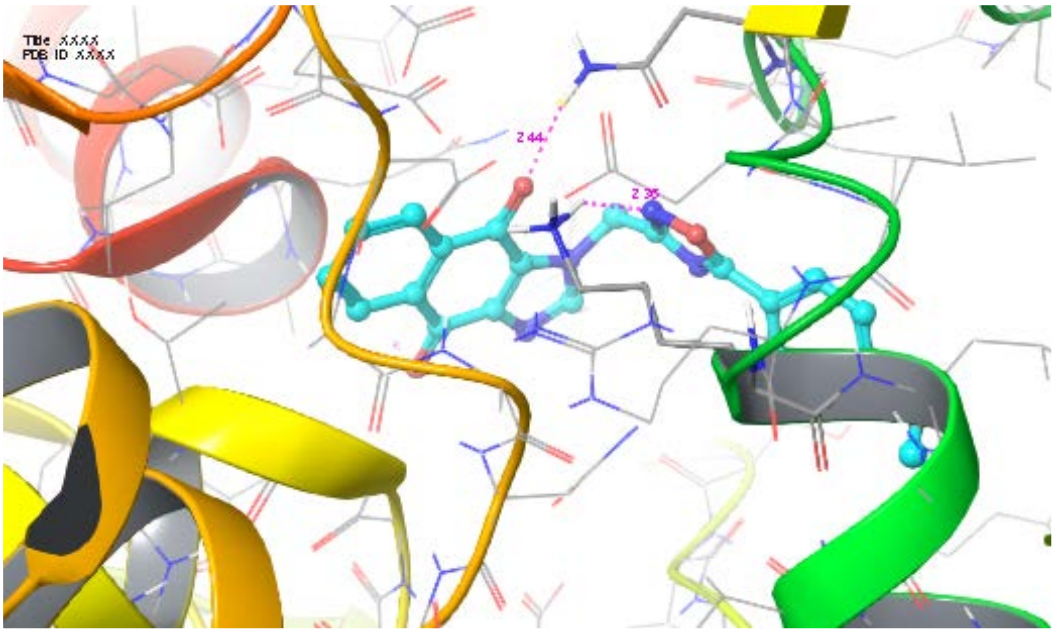


Figure 2: 3D interaction of compound 10d with EGFR

EXPERIMENTAL

General information

Melting points were reported on a Stuart SMP10 digital melting point apparatus and were uncorrected. ESI (electron spray ionization) mass spectra (ionizing voltage of 70 eV) were obtained using a Shimadzu QP5050A quadrupole-based mass spectrometer. Mass spectral data are given as m/z (Intensity). NMR spectra were recorded on a Bruker Ascend^{R} 400 (^{1}H 400,^{13}C 100 MHz) spectrometer. ^{1}HNMR spectra were run at 400 MHz and ^{13}C spectra were run at 100 MHz in deuterated $CDCl_3$, DMSO-d6 chemical shifts are expressed in δ values (ppm) using the solvent peak as an internal standard as TMS. All coupling constant (J) values are given in hertz. The abbreviations used are as follows s, singlet; d, doublet; m, and multiplet. Thin layer chromatography routinely monitored reaction progress on silica gel precoated F254 Merck plates. Unless otherwise noted all solvents and reagents were commercially available and used without further purification.

Synthesis of 2,3-Diamino-1,4-naphthoquinone (4a):[25]

5 gr of 2,3-dichloro-1,4-naphthoquinone (22 mmol) was dissolved in 100 mL of acetonitrile and 8.3 g of potassium phthalimide (44.6 mmol,) was added to the mixture. The reaction mixture was refluxed with stirring for 12 hours under a nitrogen atmosphere. After cooling to room temperature, yellow solid was collected using suction filtration and further dried under vacuum. The dried yellow solid was transferred to a round-bottom flask containing distilled water. 5 mL of hydrazine hydrate was added to the flask and the mixture was stirred for 12 hours at 60 °C. Upon cooling to room temperature, the dark blue powder was obtained as a final product (**3a**) after filtration, which was further purified by recrystallization from ethanol. The yield of the reaction was 3.73 g (90%). M.p.: 230 -232 °C.

Synthesis of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (6a): [25]

A mixture of 3.73g of **4a** (19.84 mmol) and 10 mL of formic acid in distilled water (50 mL) was refluxed with stirring for 5 hours. After cooling to room temperature, the pH of the solution was adjusted to 9 with the addition of 30% ammonium hydroxide. The dark yellow powder **6a** was obtained in a yield of 4.36 g (83%) after repeated filtration and washing with distilled water. M.p.: 368-370 °C.

2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)acetonitrile (8a):[25]

To a solution of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (**3a**) (298 mg, 1.5 mmol) in dimethyl sulfoxide (DMSO) (1.5 mL), potassium hydroxide (148 mg, 2.5 mmol) was added. The resulted mixture was stirred intensely for 15 min at room temperature under N_2 atmosphere. After addition of relative 2-bromoacetonitrile (**7a**) (2.5 mmol), the mixture was kept stirring until the reaction was completion monitored by TLC. Water was added and the precipitate was filtered, washed with water, and dried *in vacuo* to obtain the corresponding product **8a**. Yield 32%. M.p.: 212 °C.

General procedure for the one-pot synthesis of 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,4-oxadiazole hybrids (10a-l):

To a 100 mL of round bottom flask containing 10 mL of dry DCM, compound **8a** (0.001 mol), $NH_2OH.HCl$ (0.0015 mol) and Et_3N (0.0015 mol) were added and stirred at RT for 7 hours. Then, the several aromatic carboxylic acids (0.001mol) and Vilsmeier reagent (0.002 mol) were added and stirring was continued for an additional 7 hours at same temperature. The progress of the reaction, as analyzed by TLC, then the reaction mixture was washed with saturated $NaHCO_3$ (20 ml) and brine (20 mL) solutions. Later, the organic solvent was reduced under rotary evaporator to get crude product which was finally purified

by silica gel column chromatography (60-120 mesh size) using (2:8) MeOH/DCM to get the pure 1*H*-naphtho[2,3-*d*]imidazole-4,9-dione-1,2,4-oxadiazole hybrids (**10a-10l**).

1-((5-phenyl-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10a): 61% yield; M.p.: 242-244 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.15 (d, *J* = 4.5.0 Hz, 2H), 7.62 (d, *J* = 2.8 Hz, 2H), 7.52 (s, 1H), 7.45–7.38 (m, 2H), 7.01 (t, *J* = 8.1 Hz, 3H), 5.85 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 180.35, 176.95, 173.40, 163.42, 138.55, 136.52, 135.12, 133.14, 130.86, 128.26, 127.48, 125.51, 124.75, 124.65, 122.42, 45.23 ppm; MS (ESI) m/z: 357 [M+H]$^+$; CHN analysis for $C_{20}H_{12}N_4O_3$; Calculated (%): C, 67.41; H, 3.39; N, 15.72; Found (%): C, 67.38; H, 3.38; N, 15.74.

1-((5-(p-tolyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10b): 65% yield; M.p.: 246-248 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.22–8.18 (m, 2H), 7.79 (d, *J* = 1.5 Hz, 2H), 7.56 (s, 1H), 7.53–7.48 (m, 2H), 7.31 7.28 (m, 2H), 5.89 (s, 2H), 2.33 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 181.42, 176.21, 174.25, 162.65, 143.12, 139.46, 138.59, 134.21, 130.80, 129.51, 128.88, 128.03, 127.91, 123.45, 44.59, 25.36 ppm; MS (ESI) m/z: 371 [M+H] $^+$; CHN analysis for $C_{21}H_{14}N_4O_3$; Calculated (%): C, 68.10; H, 3.81; N, 15.13; Found (%): C, 68.07; H, 3.79; N, 15.11.

1-((5-(3,5-dimethylphenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione(10c): 67% yield; M.p.: 251-253 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.29 (d, *J* = 3.9 Hz, 2H), 7.79 (d, *J* = 1.7 Hz, 2H), 7.71 (s, 1H), 7.31–7.28 (m, 2H), 7.19 (s, 1H), 5.90 (s, 2H), 2.34 (s, 6H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 184.43, 174.72, 173.09, 162.70, 138.59, 138.12, 136.51, 133.15, 130.61, 128.94, 128.52, 127.42, 124.45, 123.28, 43.94, 24.71 ppm; MS (ESI) m/z: 385 [M+H]$^+$; CHN analysis for $C_{22}H_{16}N_4O_3$; Calculated (%): C, 68.74; H, 4.20; N, 14.58; Found (%): C, 68.71; H, 4.18; N, 14.56.

1-((5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10d): 65% yield; M.p.: 252-254 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.30 (d, *J* = 1.5 Hz, 2H), 7.82–7.78 (m, 2H), 7.62–7.58 (m, 3H), 7.10–7.07 (m, 2H), 5.92 (s, 2H), 3.95 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 187.40, 176.45, 173.25, 162.71, 161.42, 140.56, 138.59, 135.26, 130.19, 129.23, 127.08, 125.26, 123.76, 116.62, 58.04, 56.25 ppm; MS (ESI) m/z: 387 [M+H]$^+$; CHN analysis for $C_{21}H_{14}N_4O_4$; Calculated (%): C, 65.28; H, 3.65; N, 14.50; Found (%): C, 65.26; H, 3.62; N, 14.47.

1-((5-(3,5-dimethoxyphenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10e): 63% yield; M.p.: 260-262 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.28 (d, *J* = 1.8 Hz, 2H), 7.75 (d, *J* = 1.2 Hz, 2H), 7.69 (s, 1H), 7.20 (s, 2H) 6.77 (s, 1H), 5.95 (s, 2H), 3.97 (s, 6H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 184.52, 174.59, 174.04, 162.75, 160.18, 138.51, 137.87, 133.45, 129.12, 128.58, 126.12, 123.45, 107.31, 103.78, 58.04, 47.26 ppm; MS (ESI) m/z: 417 [M+H]$^+$; CHN analysis for $C_{22}H_{16}N_4O_5$; Calculated (%): C, 63.46; H, 3.87; N, 13.46; Found (%): C, 63.43; H, 3.86; N, 13.47.

1-((5-(4-chlorophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10f) : 63% yield; M.p.: 247-249 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.32- 8.27 (m, 2H), 7.85-7.78 (m, 2H), 7.63 (s, 1H), 7.59-7.54 (m, 2H), 7.48-7.45 (m, 2H), 5.91 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 183.41, 173.86, 175.43, 164.69, 141.51, 138.33, 137.21, 133.01, 131.29, 129.26, 129.05, 128.96, 128.72, 125.41, 46.29 ppm; MS (ESI) m/z: 391 [M+H]$^+$; CHN analysis for $C_{20}H_{11}ClN_4O_3$; Calculated (%): C, 61.47; H, 2.84; N, 14.34; Found (%): C, 61.44; H, 2.83; N, 14.36.

1-((5-(3,5-dichlorophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10g): 64% yield; M.p.: 254-256 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.32 (d, *J* = 6.0 Hz, 2H), 7.83 (d, *J* = 6.5 Hz, 2H), 7.69 (s, 1H), 7.50–7.43 (m, 3H), 5.93 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 186.42, 174.26, 173.09, 163.68, 140.51, 138.57, 134.61, 134.49, 130.15, 129.29, 129.01, 128.86, 128.26, 125.49, 47.29 ppm; MS (ESI) m/z: 426 [M+H]$^+$; CHN analysis for $C_{20}H_{10}Cl_2N_4O_3$; Calculated (%): C, 56.49; H, 2.37; N, 13.18; Found (%): C, 56.46; H, 2.36; N, 13.16.

1-((5-(4-bromophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1H-naphtho[2,3-d]imidazole-4,9-dione (10h): 62% yield; M.p.: 269-271 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.23–8.18 (m, 2H), 7.65 – 7.60 (m, 2H), 7.53 (s, 1H), 7.43–7.38 (m, 2H), 7.33–7.29 (m, 2H), 5.79 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 184.19, 173.82, 171.49, 162.29, 141.57, 138.27, 133.21, 131.51, 130.81, 129.21, 128.58, 126.75, 124.48, 123.62, 45.08 ppm; MS (ESI) m/z: 435 [M+H]$^+$ & 437 [M+3H]$^+$; CHN analysis for $C_{20}H_{11}BrN_4O_3$; Calculated (%): C, 55.19; H, 2.55; N, 12.87; Found (%): C, 55.17; H, 2.51; N, 12.84.

1-((5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10i): 64% yield; M.p.: 245-247 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.42–8.36 (m, 2H), 7.87–7.80 (m, 2H), 7.70–7.65 (m, 2H), 7.55 (s, 1H), 7.19–7.13 (m, 2H), 5.78 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 186.25, 176.17, 173.38, 166.29, 162.73, 140.18, 137.29, 136.27, 131.41, 129.96, 129.29, 126.47, 125.43, 117.67, 45.91 ppm; MS (ESI) m/z: 375 [M+H]$^+$; CHN analysis for $C_{20}H_{11}FN_4O_3$; Calculated (%): C, 64.17; H, 2.96; N, 14.97; Found (%): C, 64.15; H, 2.93; N, 14.95.

1-((5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-dione (10j): 64% yield; M.p.: 257-259 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.97–7.92 (m, 4H), 7.85–7.81(m, 4H), 7.67 (s, 1H), 5.82 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 187.29, 174.52, 172.43, 162.70, 150.25, 140.29, 138.51, 133.21, 130.81, 129.32, 128.51, 128.12, 126.13, 124.19, 46.16 ppm; MS (ESI) m/z: 402 [M+H]$^+$; CHN analysis for $C_{20}H_{11}N_5O_5$; Calculated (%): C, 59.85; H, 2.76; N, 17.45; Found (%): C, 59.81; H, 2.77; N, 17.42.

4-(3-((4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)methyl)-1,2,4-oxadiazol-5-yl)benzonitrile (10k): 65% yield; M.p.: 251-253 ºC; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.95–7.89 (m, 2H), 7.79–7.64 (m, 6H), 7.63(s, 1H), 5.82 (s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 184.29, 176.90, 175.43, 163.70, 140.56, 136.59, 135.21, 133.73, 130.12, 129.93, 129.39, 128.23, 125.47, 118.12, 114.92, 45.29 ppm; MS (ESI) m/z: 382 [M+H]$^+$; CHN analysis for $C_{21}H_{11}N_5O_3$; Calculated (%): C, 66.14; H, 2.91; N, 18.36; Found (%): C, 66.11; H, 2.88; N, 18.33.

5-(3-((4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazol-1-yl)methyl)-1,2,4-oxadiazol-5-yl)isophthalonitrile (10l): 66% yield; M.p.: 259-261 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 7.5 Hz, 2H), 8.21 – 8.18 (m, 2H), 8.12 (s, 1H), 7.85 (d, *J* = 2.6 Hz, 2H), 7.81 (s, 1H), 5.98(s, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.29, 176.75, 173.04, 163.70, 141.56, 136.59, 133.21, 132.69, 130.81, 129.12, 126.55, 125.47, 124.26, 123.05, 119.73, 46.73 ppm; MS (ESI) m/z: 407 $[M+H]^+$; CHN analysis for $C_{22}H_{10}N_6O_3$; Calculated (%): C, 65.03; H, 2.48; N, 20.68; Found (%): C, 65.01; H, 2.45; N, 20.66.

MTT assay

Individual wells of a 96-well tissue culture microlitre plate were inoculated with 100 µL of complete medium containing 1×104 cells. The plates were incubated at 37 °C in a humidified 5% CO_2 incubator for 18 hours before the experiment. After medium removal, 100 µL of fresh medium containing the test compounds and doxorubicin at different concentrations such as 0.5, 1, and 2 µM were added to each well and incubated at 37 °C for 24 hours. Then the medium was discarded and replaced with 10 µL MTT dye. Plates were incubated at 37 °C for 2 hours. The resulting formazan crystals were solubilized in a 100 µL extraction buffer. The optical density (O.D) was read at 570 nm with a microplate reader (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 protein data bank which is having PDB id 1SA0. Ligand and water are removed from the protein and calculated Gasteiger charges after the addition of polar hydrogens. The ligands are drawn using chem 3D and saved as .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.

CONCLUSION

Herein, we, described the synthesis of some new 1,2,4 oxadiazole–1*H*-naphtho[2,3-*d*]imidazole-4,9-dione hybrids (**10a-10l**) using Gabriel and Vilsmeier reactions as key approaches. Amongst the series, compound **10d, 10j, 10k** has shown superior potency against all the cell lines than the standard drug etoposide with IC_{50} values in the range of 0.082 to 1.96 µM. As well, the compounds **10e**, **10g**, and **10l** showed the most promising activity when compared with the Etoposide. The results of molecular docking studies of compounds **10d, 10j, 10k** as Tubulin targeting agents were also found to be consistent with the observed IC_{50} data.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest, financial or academic for publication of this work.

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