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# Synthesis, Characterization, Antimalarial and Anticancer Activities of Few New Amino Analogues of 1,4-Naphthoquinone

S.S. Sanjay[1,2,✉], Shashiprabha[2,3], K. Shridhara[2,4], K. Nagarajan[2,5], H. Sivaramakrishnan[6] and B. Arun[7]

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## ABSTRACT

Present study involves the synthesis, characterization, antimalarial and anticancer activities of some novel substituted amino analogues of 1,4-naphthoquinone. The chloro group present in the key starting materials like 2,3-dichloro-1,4-naphthoquinone (**1**) and 2-chloro-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (**2**) was replaced by substituted amines. These analogues were isolated, purified and screened for antimalarial and anticancer activities. A few of the novel compounds were found to possess substantial biological activity and are reasonably potent.

## KEYWORDS

Naphthoquinone derivatives, Synthesis, Characterization, Antimalarial activity, Anticancer activity.

***Author affiliations:***

[1]Department of Chemistry, Sri Dharmasthala Manjunatheshwara Institute of Technology (Affiliated to Visvesvaraya Technological University, Belagavi), Ujire-574240, India
[2]Alkem Laboratories Limited, Research & Development Centre, Peenya Industrial Area, Bangalore-560058, India
[3]PG Department of Chemistry, Sri Dharmasthala Manjunatheshwara College (Autonomous), Ujire-574240, India
[4]Technical Coordinator, ArkGen Pharma Private Limited, Peenya Industrial Area, Bangalore-560058, India
[5]#1st Floor, House No. 007, Serene Urbana, Ozone Urbana, Southe Gowdanahalli, Bangalore-562110, India
[6]Piramal Life Sciences, 1-Nirlon Complex, Off Western Express Highway, Goregaon (East), Mumbai-400063, India
[7]Chief Innovation Officer, Omni Active Health Technologies Limited, Phoenix House, T-8, A-Wing, 462-Senapati Bapat Marg, Lower Parel, Mumbai-400013, India

✉To whom correspondence to be addressed:

E-mail: sanjayss@sdmit.in

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## INTRODUCTION

Quinones play an important role in imparting biological activity to large number of molecules [1,2]. Good number of 1,4-naphthoquinone derivatives found to possess antitumor [3], antimalarial [4], antiviral [5], molluscidal [6], antileishmanial [7], antiproliferative, antibacterial and antifungal [8] activities. Incorporation of substituted amine group at 2-position of 1,4-naphthoquinone has led to the disclosure of many molecules having antifungal, antibacterial, antimalarial and anticancer activities. Moreover, it forms the molecular framework for numerous natural compounds like rifamycins [9], kinamycins [10], rifampicins [11] and streptovaricins [12]. 2,3-Dichloro-1,4-naphthoquinone (**1**) and its derivatives have shown considerable biological activity as antifungal, antibacterial, anticancer, antiplatlet, anti-inflammatory, antiallergic and anti-HIV agents [13]. The popular drug atovaquone (ATQ) was synthesized by the synthetic route, which involves the isolation and hydrolysis of intermediate 2-chloro-3-[*trans*-4-(4-chlorophenyl)-cyclohexyl]-1,4-naphthoquinone (**2**) [14]. Compound **2** has a replaceable chloro (-Cl) group attached to the naphthoquinone ring.

Calandra and Adams [15] reported the synthesis and biological activity of amino derivatives of 2-chloro-1,4-naphthoquione by treating **1** with amino acids, aminoalkanes, amino benzene, sulfonamide, amino pyridines, *etc.* Ryu *et al.* [16]

reported the amination of 2,3-dihalo-1,4-naphthoquinones with arylamines in ethanol and tested them for anticoagulant and cytotoxic activity. Lien *et al*. [17] reported the synthesis of 2-alkyl/aryl-carboxamido derivatives of 3-chloro-1,4-naphthoquinone and found them to possess antiplatelet, antiinflammatory and antiallergic activities. Van Allan *et al*. [18] reported the synthesis of a few 2-arylamino 3-chloro-1,4-napthoquinone derivatives along with isolation of a few byproducts. Gokmen and Alahmad [19] reported the synthesis and characterization of a few amino and thio(substituted) derivatives of naphthoquinones. Kiymet [20] reported the synthesis of a few novel series of 2-substituted 1,4-naphthoquinones and 2-substituted 1,4-anthraquinones, from 2,5,8-tribromo-1,4-naphthoquinone and 2,9,10-tribromo-1,4-anthraquinone, respectively. The compounds prepared had the potential to develop fingermarks, hence these compounds can be used as an effective reagents for detecting latent finger-marks in routine criminal investigations. Farahani *et al*. [21] reported the synthesis and characterization of series of 2-amino-3-(2-oxothiazol-methyl)-substituted 1,4-naphthoquinone compounds prepared by one-pot synthesis. It involves the reaction of 2-aminothiazole, 2-hydroxy-1-4-naphthoquinone and aromatic aldehydes using of nano-$SiO_2$ (20% mol). Gholampour *et al*. [22] reported the synthesis and *in vitro* cytotoxic activity of novel series of 2-amino-1,4-naphthoquinones bearing oxyphenyl moiety. Li *et al*. [23] reported the synthesis and biological activities (antiplatelet, anti-inflammatory and antiallergic) of amino, 2-alkylamino, 2-methoxy, 2-acetamido and 5,8-diacetoxy derivatives of the main compound 2,3-dichloro-5,8-dimethoxy-1,4-naphthoquinone along with 6,7-dichloro-5,8-dimethoxy-1,4-naphthoquinone. Prachayasittikul *et al*. [24] reported the synthesis and anticancer activities of series of 2-substituted amino-3-chloro-1,4-naphthoquinone derivatives. Salmon-Chemin *et al*. [25] reported the use of starting materials such as menadione, plumbagin and juglone, for the synthesis of three different series of 1,4-naphthoquinones and the correlation between redox cycling activities and *in vitro* cytotoxicity.

Based on the literature review, a few novel substituted amino analogues of 1,4-naphthoquinone are prepared. Present work involves the synthesis, purification, characterization and the biological activities of prepared novel compounds. The chloro (-Cl) group present in the key starting materials **1** and **2**, was replaced by various substituted amines and isolated crude products were purified by slurry wash/recrystallization techniques column chromatography. The purified analogues **3a-m** and **4a-j** were further evaluated for their antimalarial and anticancer activities.

## EXPERIMENTAL

Present work involves the use of key starting material **1**, which was procured from Sigma-Aldrich. Compound **2** was synthesized by the novel process to prepare the renowned antimalarial drug ATQ using compound **1** [26]. All the substituted amines were procured from Sigma-Aldrich, potassium carbonate from Rankem and the commercial solvents were procured locally. These procured starting materials, reagents and solvents were used in the experiments without further purification.

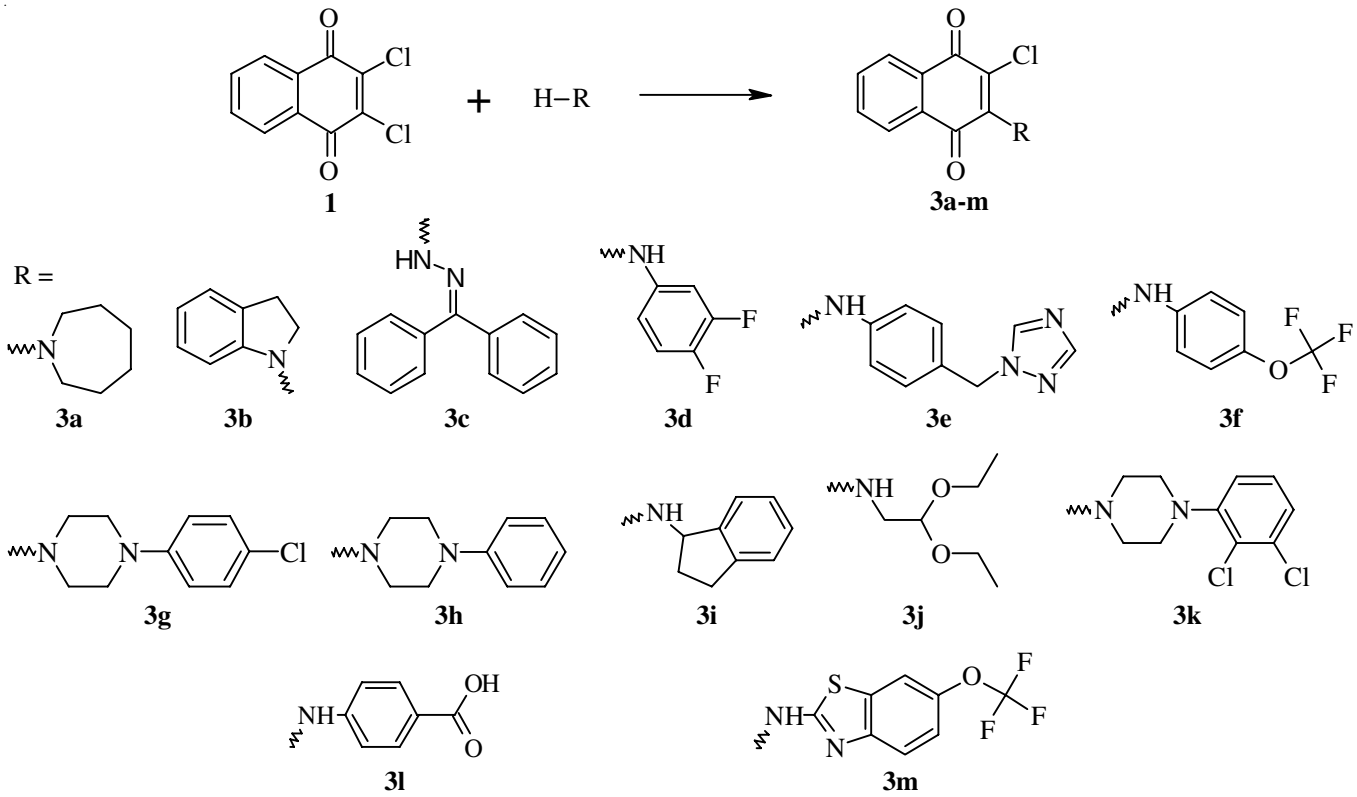
Melting points of the isolated analogues were recorded by the open capillary method and are uncorrected. $^1$H NMR spectra are recorded (in DMSO-$d_6$/$CDCl_3$) on a 300/400 MHz NMR spectrometer using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on Agilent mass spectrometer operating at 70 eV. Progress of reactions and the purity of products were checked by thin layer chromatography (TLC) using precoated silica TLC plates (Merck $^{60}F_{254}$).

**Synthesis of amino analogues of 1 (3a-f, 3j and 3l):** Compound **1** (0.50 g, 2.20 mmol), hexamethyleneimine (0.32 g, 3.30 mmol for **3a**), indoline (0.41 g, 3.52 mmol for **3b**), biphenylmethylidene hydrazone (0.69 g, 3.52 mmol for **3c**), 3,4-difluoroaniline (0.51 g, 3.96 mmol for **3d**), 4-(triazolyl-1)methylaniline (0.65 g, 3.74 mmol for **3e**), 4-trifluromethoxy-aniline (0.66 g, 3.74 mmol for **3f**), amino acetaldehyde diethyl-acetal (0.53 g, 3.96 mmol for **3j**), 4-aminomethylbenzoic acid (0.43g, 2.86 mmol for **3l**) and 6 mL methanol were stirred at 35-40 ºC for 4-6 h. After the completion of each reaction, product was isolated by filtration and dried under vacuum at 40-45 ºC for 1-2 h (**3a** = 0.51g, 81% yield, **3b** = 0.57 g, 84% yield, **3c** = 0.69 g, 81% yield, **3d** = 0.55 g, 79% yield, **3e** = 0.69 g, 86% yield, **3f** = 0.70 g, 87% yield, **3j** = 0.65 g, 92% yield and **3l** = 0.44 g, 59% yield) (**Scheme-I**).

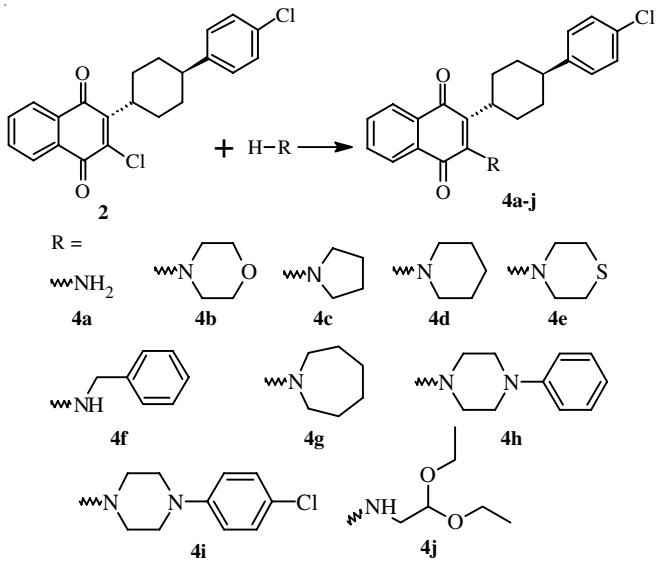
**Synthesis of amino analogues of 1 (3g-i, 3k and 3m):** Compound **1** (0.50 g, 2.20 mmol), 4-chloro phenyl piperazine (0.60 g, 3.08 mmol for **3g**), 4-phenyl piperazine (0.53 g, 3.30 mmol for **3h**), 1-aminoindane hydrochloride (0.63 g, 3.74 mmol for **3i**), 2,3-dichlorophenylpiperazine (1.01 g, 4.4 mmol for **3k**), 6-[trifluoromethoxy] benzothiazole-2-amine (1.20 g, 5.5 mmol for **3m**), $K_2CO_3$ (0.60 g, 4.40 mmol) and 10-14 mL acetonitrile were stirred at 25-30 ºC for 5-7 h. After the completion of each reaction, filtered the contents and the solvent was evaporated under reduced pressure. To the residue, added 10 mL methanol/chloroform (4:1) mixture. Stirred for 30 min at 25-30 ºC, filtered the mass under suction and dried under reduced pressure at 40-45 ºC for 1 h (**3g** = 0.76 g, 90% yield, **3h** = 0.66 g, 86% yield, **3i** = 0.41 g, 58% yield, **3k** = 0.83 g, 91% yield and **3m** = 0.64g, 69% yield) (**Scheme-I**).

**Synthesis of 2-amino-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4a):** Compound **2** (0.80 g, 2.07 mmol), 20 mL liquor ammonia and 5 mL tetrahydrofuran were stirred at 35-40 ºC for 96 h. TLC showed about 20-25% of product formation. Cooled the mass to 25-30 ºC, decanted the upper dark layer. Distilled off the solvent under reduced pressure and purified by column chromatography over neutral alumina using toluene as eluent. Product **4a** was isolated by the concentration of main fraction and dried under reduced pressure for 1 h at 40-45 ºC (0.12 g, 16% yield) (**Scheme-II**).

**Synthesis of amino analogues of 2 (4b-e):** Compound **2** (0.80 g, 2.07 mmol), morpholine (0.27 g, 3.10 mmol for **4b**), pyrrolidine (0.27 g, 3.51 mmol for **4c**), piperidine (0.29 g, 3.51 mmol for **4d**), thiomorpholine (0.38 g, 3.72 mmol for **4e**) and 8-12 mL methanol were refluxed for 36-52 h. Cooled the reaction mass to 25-30 ºC, product was isolated by filtration and dried under reduced pressure for 1 h at 40-45 ºC (**4b** = 0.19 g, 21.12% yield, **4c** = 0.29 g, 34% yield, **4d** = 0.33 g, 37% yield and **4e** = 0.47 g, 51% yield) (**Scheme-II**).




**Scheme-I:** Synthesis of amino analogues of compound **1**




**Scheme-II:** Synthesis of amino analogues of compound **2**

**Synthesis of amino analogues of 2 (4f-j):** Compound **2** (0.0 8 g, 2.07 mmol), $K_2CO_3$ (0.42 g, 3.1 mmol), benzylamine (0.55 g, 5.17 mmol for **4f**) hexamethyleneimine (0.62 g, 5.38 mmol for **4g**), phenylpiperazine {0.83 g, 5.17 mmol for **4h**), 4-chloro phenyl piperazine (1.05 g, 5.38 mmol for **4i**), amino-acetaldehyde diethylacetal (0.82 g, 6.21 mmol for **4j**) and 12-16 mL acetonitrile were heated to reflux for 30-48 h. Cooled the reaction mass to 25-30 ºC, filtered the reaction mass and distilled off the solvent under reduced pressure. To the sticky residue, added 5 mL methanol and heated to 45-50 ºC for 15 min. Filtered the hot reaction mass to isolate the product and dried under reduced pressure for 1 h at 40-45 ºC (**4f** = 0.52 g, 55% yield, **4g** = 0.29 g, 31% yield, **4h** = 0.44 g, 42% yield, **4i** = 0.39 g, 35% yield and **4j** = 0.43 g, 43% yield) (**Scheme-II**).

**2-(Azepan-1-yl)-3-chloro-1,4-naphthoquinone (3a):** m.f.: $C_{16}H_{16}NO_2Cl$, m.p.: 87-90 ºC, $^1$H NMR (300 MHz, $CDCl_3$, δ ppm): 7.95- 8.09 (2H, m), 7.61-7.71 (2H, m), 3.71-3.74 (4H, t, *J* = 6.5 Hz), 1.68-1.84 (8H, m). MS (*m/z*): 290.05 ($M^+$), LC-MS (purity): 95.47%.

**2-Chloro-3-(2,3-dihydro-1*H*-indol-1-yl)-1,4-naphthoquinone (3b):** m.f.: $C_{18}H_{12}NO_2Cl$, m.p.: 176-178 ºC, $^1$H NMR (400 MHz, $CDCl_3$): 8.17-8.19 (1H, d, *J* = 7.36 Hz), 8.07-8.09 (1H, d, *J* = 7.44 Hz), 7.71-7.77 (2H, m), 7.07-7.26 (2H, m), 6.33-6.95 (2H, m), 4.39-4.43 (2H, t, *J* = 7.8 Hz), 3.17-3.21 (2H, t, *J* = 7.7 Hz). MS (*m/z*): 308.0 ($M^+$ -2), 308.7 ($M^+$-1), 310.0 ($M^+$), 310.8 ($M^+$+1).

**2-Chloro-3-[2-(diphenylmethylidene)hydrazinyl]-1,4-naphthoquinone (3c):** m.f.: $C_{23}H_{15}N_2O_2Cl$, m.p.: 235-237 ºC, $^1$H NMR (400 MHz, $CDCl_3$): 9.18 (1H, s), 8.17-8.19 (1H, d, *J* = 7.68 Hz), 7.94-7.96 (1H, d, *J* = 7.64 Hz), 7.60-7.75 (7H, m), 7.36-7.40 (5H, m). MS (*m/z*): 387.0 ($M^+$), 388.0 ($M^+$+1), 389.0 ($M^+$+2).

**2-Chloro-3-[(3,4-difluorophenyl)amino]-1,4-naphthoquinone (3d):** m.f.: $C_{16}H_8NO_2F_2Cl$, m.p.: 263-265 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.18-8.20 (1H, d, *J* = 7.6 Hz), 8.11-8.13 (1H, d, *J* = 7.56 Hz), 7.77-7.80 (1H, t, *J* = 7.56 Hz), 7.69-7.73 (1H, t, *J* = 7.48 Hz), 7.53 (1H, bs), 7.10-7.17 (1H, m), 6.91-6.96 (1H, m), 6.82-6.84 (1H, m). MS (*m/z*): 319.9 ($M^+$), 3.18.9 ($M^+$-1), 318.0 ($M^+$-2).

**2-Chloro-3-{[4-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl]-amino}-1,4-naphthoquinone (3e):** m.f.: $C_{19}H_{13}N_4O_2Cl$, m.p.: 224-226 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.19-8.21 (1H, d, *J* = 8.0 Hz), 8.11-8.13 (1H, d, *J* = 8.0 Hz) 8.09 (1H, s), 7.99 (1H, s), 7.76-7.80 (1H, t, *J* = 7.48 Hz),7.68-7.72 (1H, t, *J*

= 7.56 Hz), 7.64 (1H, bs), 7.24-7.26 (2H, d, *J* = 8.0 Hz), 7.06-7.08 (2H, d, *J* = 8.0 Hz), 5.56 (2H, s). MS (*m/z*): 363.0 ($M^+$-1), 364.0 ($M^+$), 365.0 ($M^+$ +1), 366.1 ($M^+$ +2).

**2-Chloro-3-{[4-(trifluoromethoxy)phenyl]amino}-naphthalene-1,4-dione (3f):** m.f.: $C_{17}H_9NO_3F_3Cl$, m.p.: 206-208 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm); 8.12-8.21 (2H, m), 7.77-7.81 (1H, t, *J* = 7.5 Hz), 7.69-7.73 (1H, t, *J* = 7.6 Hz), 7.62 (1H, s), 7.19-7.22 (2H, d, *J* = 8.6 Hz), 7.08-7.10 (2H, d, *J* = 8.8 Hz). MS (*m/z*): 366.0 ($M^+$-1), 367.0 ($M^+$), 367.9 ($M^+$+1), 368.9 ($M^+$+2).

**2-Chloro-3-[4-(4-chlorophenyl)piperazin-1-yl]-1,4-naphthoquinone (3g):** m.f.: $C_{20}H_{16}N_2O_2Cl_2$, m.p.: 152-155 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.11-8.13 (1H, d, *J* = 7.3 Hz), 8.01-8.03 (1H, d, *J* = 7.3 Hz), 7.65-7.73 (2H, m), 7.23-7.25 (2H, d, *J* = 8.6 Hz), 6.92-6.94 (2H, d, *J* = 7.6 Hz), 3.77-3.78 (4H, t, *J* = 4.8 Hz), 3.32-3.35 (4H, t, *J* = 4.8 Hz). MS (*m/z*): 386.1 ($M^+$ -1), 387.0 ($M^+$), 388.0 ($M^+$ +1), 389.2 ($M^+$ +2).

**2-Chloro-3-(4-phenylpiperazin-1-yl)-1,4-naphthoquinone (3h):** m.f.: $C_{20}H_{17}N_2O_2Cl$, m.p.: 125-127 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.12-8.14 (1H, d, *J* = 7.4 Hz), 8.02-8.04 (1H, d, *J* = 7.4 Hz), 7.65-7.73 (2H, m), 7.28-7.32 (2H, t, *J* = 8.4 Hz), 6.97-6.99 (2H, d, *J* = 8.0 Hz), 6.89-6.93 (2H, t, *J* = 7.28 Hz), 3.77-3.79 (4H, t, *J* = 4.8 Hz), 3.35-3.38 (4H, t, *J* = 4.8 Hz). MS (*m/z*): 352.1 ($M^+$ -1), 353.1 ($M^+$), 354.1 ($M^+$+1), 355.1 ($M^+$+2).

**2-Chloro-3-(2,3-dihydro-1*H*-inden-1-ylamino)-1,4-naphthoquinone (3i):** m.f.: $C_{19}H_{14}NO_2Cl$, m.p.: 177-180 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.17-8.19 (1H, d, *J* = 7.76 Hz), 8.03-8.05 (1H, d, *J* = 7.64 Hz), 7,72-7.76 (1H, t, *J* = 7.56 Hz), 7.61-7.65 (1H, t, *J* = 7.6 Hz), 7.24-7.37 (4H, m), 6.26 (1H, m), 6.09-6.14 (1H, m), 3.03-3.11 (1H, m), 2.90-2.98 (1H, m), 2.67-2.76 (1H, m), 2.00-2.17 (1H, m). MS (*m/z*): 323.8 ($M^+$), 325.8 ($M^+$+2).

**2-Chloro-3-[(2,2-diethoxyethyl)amino]-1,4-naphthoquinone (3j):** m.f.: $C_{16}H_{18}NO_4Cl$, m.p.: 62-65 ºC, $^1$H NMR (300 MHz, $CDCl_3$, δ ppm): 8.13-8.16 (1H, d, *J* = 7.8 Hz), 8.02-8.05 (1H, d, *J* = 7.5 Hz), 7.60-7.75 (2H, m), 6.29 (1H, bs), 4.67-4.70 (1H, t, *J* = 5.4 Hz), 3.98-4.01 (2H, t, *J* = 5.7 Hz), 3.71-3.81 (2H, m), 3.54-3.65 (2H, m), 1.23-1.28 (6H, t, *J* = 6.9 Hz). MS (*m/z*): 324.05 ($M^+$), LC-MS (purity): 95.28%.

**2-Chloro-3-[4-(2,3-dichlorophenyl)piperazin-1-yl]-1,4-naphthoquinone (3k):** m.f.: $C_{20}H_{15}N_2O_2Cl_3$, m.p.: 173-175 ºC, $^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm): 7.97-8.02 (2H, m), 7.79-7.86 (2H, m), 7.33-7.35 (2H, d, J = 4.8 Hz), 7.20-7.23 (1H, t, *J* = 4.8 Hz), 3.71 (4H, bs), 3.16-3.32 (4H, m). MS (*m/z*): 421.0 ($M^+$), LCMS (purity): 100%.

**4-[(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-amino]benzoic acid (3l):** m.f.: $C_{17}H_{10}NO_4Cl$, m.p.: 245-247 ºC, $^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm): 12.81-12.82 (1H, bs), 8.07-8.12 (1H, t, *J* = 6.9 Hz), 7.91-7.98 (2H, m), 7.72-7.88 (4H, m), 7.40-7.43 (2H, d, *J* = 8.1 Hz), 5.01-5.03 (2H, d, *J* = 6.9 Hz). MS (*m/z*): 342.0 ($M^+$).

**2-Chloro-3-{[6-(trifluoromethoxy)-1,3-benzothiazol-2-yl]amino}-1,4-naphthoquinone (3m):** $C_{18}H_8N_2O_3SF_3Cl$, m.p.: 171-173 °C, $^1$H NMR (400 MHz, DMSO-$d_6$): 8.01-8.08 (2H, m), 7.84-7.91 (2H, m), 7.31-7.33 (3H, m). MS (*m/z*): 423.0 ($M^+$ -1), 424.0 ($M^+$), 425.0 ($M^+$+1), 426.0 ($M^+$+2).

**2-Amino-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4a):** m.f.: $C_{22}H_{20}NO_2Cl$, m.p.: 263-265 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 8.06-8.08 (1H, d, *J* = 8.0 Hz), 8.00-8.02 (1H, d, *J* = 8.0 Hz), 7.68-7.71 (1H, t, *J* = 8.0 Hz), 7.57-7.61 (1H, t, *J* = 8.0 Hz), 7.26-7.28 (2H, d, *J* = 8.0 Hz), 7.16-7.88 (2H, d, *J* = 8.0 Hz) 5.22 (2H, s), 2.75-2.81 (1H, m), 2.62-2.68 (1H, m), 2.16-2.23 (2H, m), 2.00-2.03 (2H, m), 1.79-1.82 (2H, m), 1.51-1.58 (2H, m). MS (*m/z*): 365.31 ($M^+$), 366.23 ($M^+$+1), 367.32 ($M^+$+2).

**2-Morpholino-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4b):** mf.: $C_{26}H_{26}NO_3Cl$, m.p.: 194-196 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.98-8.03 (2H, m), 7.64-7.71 (2H, m), 7.27-7.29 (2H, d, *J* = 8.0 Hz), 7.18-7.20 (2H, d, *J* = 8.4 Hz), 3.84-3.87 (4H, t, *J* = 4.6 Hz), 3.32-3.34 (4H, t, *J* = 4.6 Hz), 2.97-3.03 (1H, m), 2.67-2.73 (1H, m), 2.33-2.43 (2H, m), 2.00-2.03 (2H, m), 1.70-1.73 (2H, m), 1.44-1.54 (2H, m). MS (*m/z*): 436.1 ($M^+$), 437.0 ($M^+$+1), 438.2 ($M^+$+2).

**2-Pyrrolidino-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4c):** m.f.: $C_{26}H_{26}NO_2Cl$, m.p.: 168-171 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.96-7.98 (1H, d, *J* = 7.6 Hz), 7.82-7.84 (1H, d, *J* = 7.56 Hz), 7.61-7.65 (1H, t, *J* = 7.48 Hz), 7.52-7.56 (1H, t, *J* = 7.52 Hz), 7.24-7.26 (2H, d, *J* = 8.4 Hz), 7.16-7.18 (2H, d, *J* = 8.4 Hz), 3.68-3.72 (4H, m), 2.68-2.74 (1H, m), 2.45-2.62 (3H, m), 1.92-2.01 (6H, m), 1.73-1.75 (2H, m), 1.39-1.48 (2H, m). MS (*m/z*): 420.1 ($M^+$), 421.1 ($M^+$+1), 422.1 ($M^+$+2).

**2-Piperidino-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4d):** m.f.: $C_{27}H_{28}NO_2Cl$, m.p.: 192-194 ºC, $^1$H NMR (300 MHz, $CDCl_3$, δ ppm): 7.89-8.00 (2H, m), 7.54-7.62 (2H, m), 7.19-7.22 (2H, *J* = 9.0 Hz), 7.11-7.14 (2H, d, *J* = 9.0 Hz), 3.26-3.27 (4H, m), 3.13-3.18 (6H, m), 1.62-2.97 (10H, m). MS (*m/z*): 434.1 ($M^+$), 435.2 ($M^+$+1), 437.1 ($M^+$+2).

**2-Thiomorpholino-3-[*trans*-4-(4-chlorophenyl)-cyclohexyl]-1,4-naphthoquinone (4e):** m.f.: $C_{26}H_{26}NO_2SCl$, m.p.: 223-225 ºC, $^1$H NMR (300 MHz, $CDCl_3$, δ ppm): 8.00-8.06 (2H, m), 7.66-7.73(2H, m), 7.29-7.32 (2H, d, *J* = 8.4 Hz), 7.20-7.22 (2H, d, *J* = 8.4 Hz), 3.51-3.55 (4H, t, *J* = 4.8 Hz), 3.20-3.23 (1H, m), 3.00-3.08 (1H, m), 2.81-2.84 (4H, t, *J* = 4.8 Hz), 2.68-2.76 (1H, m), 1.46-2.44 (8H, m).

**2-Benzylamino-3-[*trans*-4-(4-chlorophenyl) cyclohexyl]-naphthalene-1,4-dione (4f):** m.f.: $C_{29}H_{26}NO_2Cl$, m.p.: 209-211 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.88-7.91 (2H, m), 7.66-7.79 (2H, m), 7.22-7.40 (8H, m), 7.10-7.12 (2H, d, *J* = 8.44 Hz), 4.69-4.71 (2H, d, *J* = 6.92 Hz), 2.65-2.70 (1H, m), 2.20-2.29 (2H, m), 1.63-1.66 (2H, m), 1.44-1.46 (2H, m), 1.02-1.06 (2H, m). MS (*m/z*): 456.0 ($M^+$), 457.2 ($M^+$+1), 458.2 ($M^+$+2).

**2-(Azepan-1-yl)-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4g):** m.f.: $C_{28}H_{30}NO_2Cl$, m.p.: 196-198 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.97-8.03 (2H, m), 7.63-7.69 (2H, m), 7.26-7.28 (2H, d, *J* = 8.4 Hz), 7.18-7.20 (2H, d, *J* = 8.4 Hz), 3.22-3.25 (5H, m), 2.67-2.73 (1H, t, *J* = 12.1 Hz), 2.31-2.41 (2H, m), 2.03 (2H, m), 1.75 (9H, s), 1.48-1.71 (3H, m). MS (*m/z*): 448.2 ($M^+$), 449.2 ($M^+$+1), 450.1 ($M^+$+2).

**2-(4-Phenylpiperazin-1-yl)-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4h):** m.f.: $C_{32}H_{31}N_2O_2Cl$, m.p.: 140-142 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.99-8.10 (2H, m), 7.64-7.71 (2H, m), 7.16-7.33 (7H, m), 6.89-7.02 (2H, m), 3.49-3.52 (4H, t, *J* = 4.76 Hz), 3.34-

3.36 (4H, t, *J* = 4.72 Hz), 3.01-3.07 (1H, m), 2.66-2.72 (1H, m), 2.32-2.45 (2H, m), 1.44-2.03 (6H, m). MS (*m/z*): 511.2 ($M^+$), 512.2 ($M^+$+1), 513.1 ($M^+$+2).

**2-[4-(4-Chlorophenyl)piperazin-1-yl]-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4i):** m.f.: $C_{32}H_{30}N_2O_2Cl_2$, m.p.: 212-214 ºC, $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 7.92-8.02 (2H, m), 7.75-7.77 (2H, m), 7.60-7,69 (2H, m), 7.44-7.46 (2H, d, *J* = 7.68 Hz), 7.09-7.21 (4H, m), 3.10-3.90 (8H, m), 2.70-2.73 (1H, m), 2.59-2.66 (1H, m), 1.47-2.51 (8H, m). Mass: *m/z*: 545.2 ($M^+$), 546.2 ($M^+$+1), 547.2 ($M^+$+2).

**2-[(2,2-Diethoxyethyl)amino]-3-[*trans*-4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4j):** m.f.: $C_{28}H_{32}NO_4Cl$, m.p.: 108-110 ºC, $^1$H NMR (300 MHz, $CDCl_3$, δ ppm): 7.97-8.05 (2H, m), 7.56-7.71 (2H, m), 7.26-7.28 (2H, d, *J* = 7.2 Hz), 7.16-7.19 (2H, d, *J* = 8.4 Hz), 5.82-5.85 (1H, t, *J* = 5.4 Hz), 4.64-4.67 (1H, t, *J* = 5.4 Hz), 3.71-3.78 (2H, m), 3.55-3.65 (4H, m), 2.70-2.76 (2H, m), 2.43-2.48 (2H, m), 1.74-2.02 (4H, m), 1.46-1.56 (2H, m), 1.25-1.33 (6H, t, *J* = 12.6 Hz). MS (*m/z*): 482.25 ($M^+$). LC-MS (purity): 100%.

**Antimalarial activity:** Assessment of *in vitro* antimalarial activity was done using the test model, *in vitro Plasmodium falciparum* (strain 3D7). All the analogues were evaluated for antimalarial activity against 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *Plasmodium falciparum* by employing Malaria SYBR Green-I nucleic acid staining dye based fluorescence (MSF) assay [27]. The stock (5 mg/mL) solution was prepared using the solvent dimethylsulphoxide and test dilutions are prepared in culture medium (RPMI-1640-FBS). Chloroquine-diphosphate (CQ) and ATQ were being used as reference drugs for the activity evaluation.

**Assessment methodology:** 50 µL of culture medium was dispensed in 96 well plate followed by the addition of 50 µL of highest concentration of test compounds (in duplicate wells) in row B. Subsequent two fold serial dilutions are prepared and finally 50 µL of 1.0% parasitized cell suspension containing 0.8% parasitaemia was added to each well except 4 wells in row A had received non parasitized erythrocyte suspension. The plates are incubated at 37 ºC in carbon dioxide incubator in an atmosphere of 5% $CO_2$ and air mixture for about 72 h. Later 100 µL of lysis buffer containing 2x concentration of SYBR Green-I (nitrogen) was added to each well and incubated for 1 h at 37 ºC. The plates were evaluated at 485 ± 20 nm of excitation and 530 ± 20 nm of emission for relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLX800, BIOTEK).

**Cytotoxicity assay:** The cytotoxicity estimation of the synthesized compounds was done using Vero cell line (C1008: Monkey kidney fibroblast) [28]. The cells were incubated with compound dilutions for 72 h and MTT was used as the reagent for the estimation of cytotoxicity. 50% cytotoxic concentration ($CC_{50}$) was determined by employing nonlinear regression analysis of dose response curves using pre-programmed excel spreadsheet.

**Anticancer activity:** Human tumor cell lines Panc-1 (pancreatic cancer), HCT 116 (colorectal cancer), ACHN (renal cell carcinoma), Calu-1 (lung carcinoma) were grown in Minimal Essential Media with Eagle's Basal salts (MEM-EBS) obtained from AMIMED (BioConcept, Switzerland), H460 (Small Cell Lung Cancer) were cultured in RPMI 1640 (AMIMED, BioConcept, Switzerland). All the tumor cell lines were supplemented with 10% of fetal bovine serum (FBS) (GIBCO), 1% of penicillin/streptomycin (Sigma) and 1% of *anti-anti* (GIBCO) and were grown in T-175 tissue culture flasks (Nunc). MCF-10A non-tumourigenic cell line was cultured in Mammary Epithelial Basal Medium (MEBM) with all standard conditions (Lonza, Catalog. No.CC-3150). All the cells were grown at 37 ºC with 5% $CO_2$. Cells were passaged at 80-90% confluence. Adherent cells were trypsinized using Trypsin-EDTA (Sigma) and maintained. All the cell lines were procured from ATCC (Rockville, USA).

**Sample preparation:** All the compounds were weighed accurately and dissolved in specific quantity of DMSO to give a required stock solution of 20 mmol. Eight different concentrations of the compound were prepared by serial dilution of stock solution finally resulting in a (compared to the test concentration) 200-fold lower concentration. Each compound was tested at 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10 and 30 µm. Every concentration was evaluated in duplicates. All the analogues of the present work were taken through the similar preparations and the activity aspects were estimated.

**The $IC_{50}$ determination:** Cells were plated in 96-well flat-bottom microtiter plates at a cell density of 3000 cells/well. After 24 h of recovery period to allow the cells to resume exponential growth, the compound was added at 8 concentrations in duplicates for 48 h. Post 48 h of incubation, cell toxicity was determined by CCK-8 reagent (Dojindo Molecular Technologies, Inc, Japan). The 5 µL/well CCK-8 reagent was added and plates were incubated for 2 h. The synthesized compounds induced anti-proliferation/toxicity were determined by measuring the absorbance on Tecan Sapphire multiflourescence micro plate reader (Tecan, Germany, GmbH) at a wavelength of 450 nm, corrected to 650 nm and normalized to controls.

$$\text{Cell growth inhibition (\%)} = \frac{\text{OD of vehicle control} - \text{OD of treated cells}}{\text{OD of vehicle control}} \times 100$$

**THP-1 assay protocol:** The human monocytic cell line THP-1 (ATCC, Manassas, USA) was maintained in RPMI-1640 (Bioconcept) supplemented with 10% FBS (GIBCO). Prior to LPS-stimulation, 25,000 cells per well were cultured for 24 h in the presence of 10 ng/mL of phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) to enhance their response to activation stimuli [29]. These cells were then stimulated with 1.0 µg/mL LPS (Sigma-Aldrich) for 24 h in presence or absence of compound doses at 0.5% DMSO concentration. At the end of 24 h, the cell supernatant was used for cytokine measurements and the cells used for determining the toxic effects of the compounds by the exposure to CCK-8 (DoJindo). For all experiments, the THP-1 monocytes were used only up to passage and utilized within two months post revival of a new vial.

## RESULTS AND DISCUSSION

The chloro group present in the key starting materials **1** (**Scheme-I**) and **2** (**Scheme-II**) was replaced by substituted amines to prepare the corresponding novel analogues.

Mild reaction conditions were sufficient for the synthesis of substituted amino analogues of compound **1** and the rate of reaction was extremely fast with good yield. Whereas drastic reaction conditions were required for the synthesis of amino analogues of compound **2** and the reaction was sluggish, in many cases reaction did not go for completion. The average yield observed for the amino analogues of compound **1** was 80.23%, similarly it was found to be 36.5% for the amino analogues of compound **2**. This behaviour leading to low yield can be linked to substantial steric hindrance offered by compound **2**, which was not observed in the case of compound **1**. All the synthesized analogues were characterized by spectral analysis.

The mass spectrum of compound **3a** showed an intense molecular ion peak with *m/z* value of 290.05 ($M^+$) with molecular weight of 289.75 ($C_{16}H_{16}NO_2Cl$). The chromatographic purity of compound **3a** was found to be 95.47% by LC-MS. The 300 MHz $^1$H NMR spectrum of compound **3a** showed a multiplet in the range δ 7.95-8.09 ppm, integrated for 2 protons attached to C5 and C8. The multiplet in the range δ 7.61-7.71 ppm integrated for 2 protons attached to C6 and C7. The triplet ($J$ = 6.5 Hz) in the range δ 3.71-3.74 ppm, which was integrating for 4 protons attached to C11 and C12. The remaining 8 protons attached to C13, C14, C15 and C16 respectively were resonated as multiplets in the range δ 1.68-1.84 ppm. The 300 MHz $^1$H NMR spectrum of compound **4e** showed a multiplet in the range δ 8.00-8.06 ppm integrated for 2 protons attached to C5 and C8. The multiplet in the range δ 7.66-7.73 ppm was assigned for 2 protons attached to C6 and C7. The 4 aromatic protons attached to C22, C23, C24 and C25 were resonated as 2 doublets ($J$ = 8.4 Hz each) in the range δ 7.29-7.32 ppm and 7.20-7.22 ppm. A triplet ($J$ = 4.8 Hz) in the range δ 3.51-3.55 ppm was assigned for 4 protons attached to C13 and C14. The multiplet in the range δ 3.20-3.23 ppm integrated for one proton attached to C15. The multiplet in the range δ 3.00-3.08 ppm integrated for one proton attached to C20. The triplet ($J$ = 4.8 Hz) in the range δ 2.81-2.84 ppm was assigned for 4 protons attached to C11 and C12. The multiplet in the range δ 2.68-2.76 ppm was assigned for two protons, one each attached to C16 and C17. Remaining six protons attached to C16, C17 (one proton each) and C18, C19 (two proton each) were resonated as multiplets in the range δ 1.46-2.44 ppm. Similarly, the structures of other newly synthesized analogues were also established.

**Antimalarial activity:** The $IC_{50}$ values are obtained by logistic regression analysis of dose response curves using pre-programmed excel spreadsheet. Atovaquone and chloroquine-diphosphate were used as the reference standards for the evaluation. The antimalarial screening data (Tables 1 and 2)

TABLE-1
$IC_{50}$, $CC_{50}$ AND SELECTIVITY INDEX (SI) OF ANALOGUES

| Compd. No. | $IC_{50}$ µg/mL | $CC_{50}$ µg/mL | SI | Compd. No. | $IC_{50}$ µg/mL | $CC_{50}$ µg/mL | SI |
|---|---|---|---|---|---|---|---|
| **3a** | > 200 | 31.43 | NA | **4a** | 59.93 | 36.79 | 613.88 |
| **3b** | > 200 | 4.75 | NA | **4b** | > 200 | 10.41 | NA |
| **3c** | > 200 | 31.5 | NA | **4c** | 92.09 | 41.61 | 451.84 |
| **3d** | > 200 | > 100 | NA | **4d** | > 200 | 80.03 | NA |
| **3e** | > 200 | 55.3 | NA | **4e** | 26.58 | 54.06 | 2033.86 |
| **3f** | > 200 | 96.31 | NA | **4f** | > 200 | 85.03 | NA |
| **3g** | > 200 | 6.47 | NA | **4g** | 61.92 | 73.18 | 1181.84 |
| **3h** | > 200 | 6.17 | NA | **4h** | 65.77 | 24.32 | 369.77 |
| **3i** | 35.07 | 40.41 | 1152.26 | **4i** | > 200 | 61.56 | NA |
| **3j** | > 200 | 17.84 | NA | **4j** | > 200 | 45.73 | NA |
| **3k** | > 200 | 51.29 | NA | ATQ (mean ± sd) | 0.393 ± 0.06 | 0.4 | 1019 |
| **3l** | > 200 | 19.94 | NA | CQ (mean ± sd) | 2.45 ± 1.028 | > 100 | Std |
| **3m** | > 200 | 7.1 | NA | | | | |

Note: NA = Not applicable, ATQ = Atovaquone, CQ = Chloroquine-diphosphate

TABLE-2
$IC_{50}$ AGAINST (3D7 AND K1) AND $CC_{50}$ OF ANALOGUES

| Compd. No. | $IC_{50}$ (µg/mL) against strain 3D7 | $IC_{50}$ (µg/mL) against strain K1 | $CC_{50}$ (µg/mL) | Compd. No. | $IC_{50}$ (µg/mL) against strain 3D7 | $IC_{50}$ (µg/mL) against strain K1 | $CC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|
| **3a** | > 200 | Nd | 31.43 | **4a** | 59.93 | 169.7 | 36.79 |
| **3b** | > 200 | Nd | 4.75 | **4b** | > 200 | Nd | 10.41 |
| **3c** | > 200 | Nd | 31.5 | **4c** | 92.09 | 192.63 | 41.61 |
| **3d** | > 200 | Nd | > 100 | **4d** | > 200 | Nd | 80.03 |
| **3e** | > 200 | Nd | 55.3 | **4e** | 26.58 | 100.71 | 54.06 |
| **3f** | > 200 | Nd | 96.31 | **4f** | > 200 | Nd | 85.03 |
| **3g** | > 200 | Nd | 6.47 | **4g** | 61.92 | 143.32 | 73.18 |
| **3h** | > 200 | Nd | 6.17 | **4h** | 65.77 | 80.77 | 24.32 |
| **3i** | 35.07 | 149.09 | 40.41 | **4i** | > 200 | Nd | 61.56 |
| **3j** | > 200 | Nd | 17.84 | **4j** | > 200 | Nd | 45.73 |
| **3k** | > 200 | Nd | 51.29 | ATQ (mean ± sd) | 0.39 ± 0.06 | 0.31 ± 0.05 | 0.4 |
| **3l** | > 200 | Nd | 19.94 | CQ (mean ± sd) | 2.45 ± 1.03 | 135.34 ± 28.72 | > 100 |
| **3m** | > 200 | Nd | 7.1 | | | | |

Note: Nd = Not detected, ATQ = Atovaquone, CQ = Chloroquine-diphosphate

TABLE-3
ANTICANCER ACTIVITY RESULTS OF ANALOGUES

| Compd. No. | H460 (non small cell lung cancer | HCT116 (colon cancer) | ACHN (renal cancer) | Calu1 (lung cancer) | Panc-1 (pancreatic cancer) | MCF110A (normal cells) | HTS ranking |
|---|---|---|---|---|---|---|---|
| **3a** | 4.0 | NA | NA | 9.0 | 4.0 | NA | Active |
| **3b** | 3.1 | 1.7 | 1.9 | 3.7 | 1.1 | > 10 | Active |
| **3c** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3d** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3e** | 1.1 | 0.7 | 1.0 | 1.1 | 0.7 | > 10 | Potent |
| **3f** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3g** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3h** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3i** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **3j** | 8.0 | NA | NA | 7.0 | 20.0 | T | Potent |
| **3k** | 5.0 | NA | NA | 6.0 | 6.0 | NT | Active |
| **3l** | 10.0 | NA | NA | 9.0 | 19.0 | NA | Active |
| **3m** | 3.7 | 1.7 | 3.1 | 3.4 | 1.9 | > 10 | Active |
| **4a** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4b** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4c** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4d** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4e** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4f** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4g** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4h** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4i** | > 10 | > 10 | > 10 | > 10 | > 10 | > 10 | – |
| **4j** | 21.0 | NA | NA | 30.0 | 90.0 | NA | Active |
| GEM | 92.0 | 69.0 | 81.0 | 71.0 | 70.0 | NA | Std. |

Note: GEM = Positive control gemcitabine, NA = Not applicable, T = Toxic, NT = Non toxic

of the synthesized substituted amine analogues of 1,4-naphthoquinones revealed that the compounds such as compounds **3i**, **4a**, **4c**, **4e**, **4g** and **4h** have shown substantial antimalarial activity.

**Anticancer activity:** Gemcitabine, a renowned anticancer drug was used as the positive control during the *in vitro* anticancer evaluation process. The investigation of the anticancer screening data (Table-3) of the synthesized substituted amine analogues of 1,4-naphthoquinones revealed that the compounds **3a**, **3b**, **3k**, **3l**, **3m** and **4j** were active. Moreover, compounds such as compounds **3e** and **3j** were found to be potent. Interestingly the compound **3e** was non-toxic to normal cell but compound **3j** was found toxic to normal cells.

## Conclusion

A few novel substituted amino analogues of compounds **1** and **2** were synthesized, purified, characterized and screened for antimalarial and anticancer activities. In case of derivatives of compound **1**, the synthetic pathway required mild reaction conditions where as drastic reaction conditions were required for the synthesis of analogues of compound **2**. This behaviour can be attributed to substantial steric hindrance offered by compound **2** contributing to lower yields. The investigation of the antimalarial activities of the synthesized substituted amine analogues of 1,4-naphthoquinones revealed that compounds **3i**, **4a**, **4c**, **4e**, **4g** and **4h** have shown the substantial activity. The investigation of the anticancer activities of analogues revealed that compounds **3a**, **3b**, **3k**, **3l**, **3m** and **4j** were found to be active. Moreover, the compounds such as compound **3e** and **3j** were found to be potent. Interestingly, compound **3e** was non-toxic to normal cell but compound **3j** was found to be toxic to normal cells.

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