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Design, Synthesis and Antimalarial Activity of Some New 2-Hydroxy-1,4-naphthoquinone-4-hydroxyaniline Hybrid Mannich Bases

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In this study, some novel 2-hydroxy-1,4-naphthoquinone-4-hydroxyaniline hybrid Mannich bases were designed, synthesized and evaluated for *in vitro* antimalarial activity. The design strategy of novel hybrid molecules involves fusion between the pharmacophoric moieties of lawsone (2-hydroxy-1,4-naphthoquinone, a residue from atovaquone) and Mannich substituted 4-hydroxyaniline (4-aminophenol, a residue from amodiaquine) on the basis of molecular hybridization strategy. Newly designed compounds, **5a-f** were also studied for drug-likeness assessment based on Lipinski's rule of five. All the synthesized compounds exhibited some degree of *in vitro* antimalarial activity against the chloroquine-sensitive strain (RKL-2) of *P. falciparum* at the tested dose (1 mg/mL), which was considerably less than that of the standard drug, chloroquine (0.1 mg/mL). However, compounds with propyl, **5a** (IC_{50} 0.453 µg/mL) and morpholinyl, **5f** (IC_{50} 0.391 µg/mL) substitutions showed comparatively better activity than rest of the synthesized analogues. Compound **5f** (IC_{50} 0.993 µg/mL) was found to possess higher antimalarial effectiveness than compound **5a** (IC_{50} 2.92 µg/mL) against resistant strain (RKL-9) of *P. falciparum*. The activity of these compounds against the resistant strain was also less than that of chloroquine (IC_{50} 0.299 µg/mL). From results, it is clear that compounds having substitutions like smaller alkyl groups (*n*-propyl, **5a**; isopropyl, **5b**) or saturated heterocyclic moiety (morpholinyl, **5f**) possess superior antimalarial activity in comparison to other compounds substituted with bulky alkyl (diisopropyl, **5c**; *n*-butyl, **5d**) or aryl (phenyl, **5e**) moieties. Further, since all the compounds exhibited favourable drug-like properties a reasonable correlation therefore appears to exist between their drug-likeness and antimalarial activities.

Keywords: 2-Hydroxy-1,4-naphthoquinone, 4-Hydroxyaniline, Mannich base, Hybrid, Drug-likeness, Antimalarial.

INTRODUCTION

Malaria is a life-threatening parasitic disease affecting around 300-500 million people, which causes over 1 million deaths per year globally [1]. The development of resistance of malaria parasites to most of the currently available antimalarial drugs is mainly attributed to be responsible for the global rise of malaria in recent times. Moreover, the emergence of multi-drug resistant strains of *Plasmodium falciparum* has further complicated the issues related to the chemotherapy of the disease in malaria endemic regions of the world [2]. Chloroquine (CQ, Fig. 1), a 4-aminoquinoline antimalarial was once used as a first line drug for the treatment of malaria because of its excellent clinical efficacy, limited host toxicity and cost-effectiveness. Resistance to chloroquine in *P. falciparum* malaria has seriously impaired the use of quinoline antimalarials in the treatment of malaria [3]. Novel artemisinin-based antimalarials and their combination regimens such as mefloquine-artemisinin derivatives have also currently become clinically less effective in multi-drug resistant *P. falciparum* malaria [4].

To overcome the problem of drug resistance in *P. falciparum* malaria, multiple traditional and modern approaches such as development of new analogues of the existing drugs, use of combination therapy, design of hybrid molecules are currently being adopted for the discovery and development of potent antimalarial agents [5]. The design of hybrid molecules by the covalent fusion of two or more pharmacophoric units having different mechanisms of action remains a very attractive strategy for the development of new antimalarial drugs. This novel rational drug design approach is based upon the concept of molecular hybridization. A single hybrid molecule with different or dual modes of action may be beneficial for the treatment of malaria in terms of overcoming drug resistance for showing action at multiple therapeutic targets with improved biological affinities and reduced undesired side effects. This strategy therefore not only remains therapeutically efficacious but also cost-effective, which ultimately reduces the overall drug pressure of the treatment. Some hybrid molecules comprising two distinct pharmacophoric moieties like 4-amino-quinoline-trioxane, 4-aminoquinoline-triazine, chloroquine-

ferrocene and 4- aminoquinoline-pyrimidine hybrids are under clinical trials as hybrid antimalarial agents [6-9].

Lawsone is chemically known as 2-hydroxy-1,4-naphthoquinone. Lawsone scaffold based synthetic drugs have potential for antimalarial effectiveness. Atovaquone (ATQ, Fig. 1), a 3-substituted derivative of lawsone is a clinically useful antimalarial agent. Literature reveal the antiplasmodial potential of natural and/or synthetic 2-amino substituted 1,4-naphthoquinones and 1,2-naphthoquinones. The antiparasitic activity of the quinones is exhibited by several mechanisms such as the competitive inhibition of the cytochrome bc1 complex, generation of reactive oxygen species (ROS), enzymatic inhibition (*e.g.*, glutathione reductase, dihydroorotate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase), alkylation of biomolecules *etc*. [10]. On the other hand, paracetamol (4-hydroxyacetanilide) contains a 4-hydroxyanilino moiety, which is believed to undergo Cyt. P-450 catalyzed oxidation *in vivo* to a reactive quinoneimine [11]. Amodiaquine (AQ, Fig. 1) is a 4-aminoquinoline antimalarial drug having Mannich substitution at the 4-hydroxyaniline residue in the side chain. Amodiaquine was effective against chloroquine-resistant strains of *P. falciparum*, but the clinical use of amodiaquine has been restricted because of its severe side effects like hepatotoxicity and agranulocytosis [12]. The toxicity of amodiaquine is due to the fact that 4-hydroxyanilino moiety undergoes enzymatic oxidative bioactivation to form reactive toxic quinoneimine metabolites such as amodiaquine quinoneimine (AQQI) or semiquinoneimine (AQSQI). However, its regioisomeric analogue, isoquine (ISQ, Fig. 1) showed excellent oral *in vivo* ED_{50} activity with less severe side effects as it did not undergo bioactivation [11,13,14].

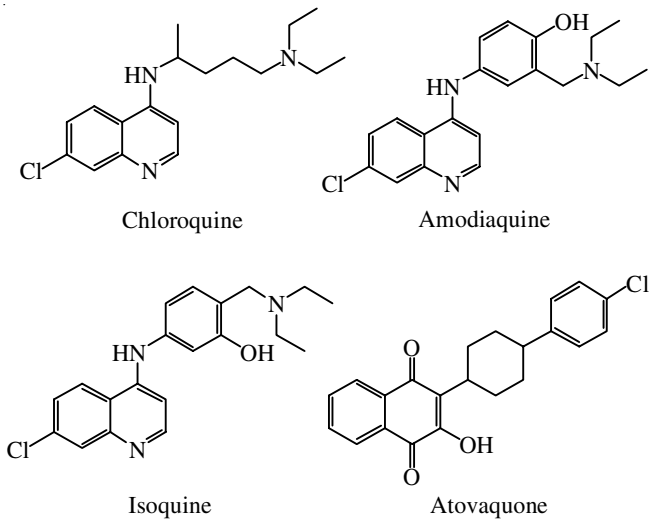


Fig. 1. Chemical structures of some potent antimalarial drugs

Keeping in view the above facts, it was thought to design some novel antimalarial derivatives through coupling between the structural scaffolds of 2-hydroxy-1,4-naphthoquinone (a residue from atovaquone) and Mannich substituted 4-hydroxyaniline (a residue from amodiaquine) based on the concept of molecular hybridization strategy (Fig. 2). Newer hybrid antimalarial agents would be potent (improved efficacy and affinity) and also active against resistant strains of *P. falciparum* malaria.

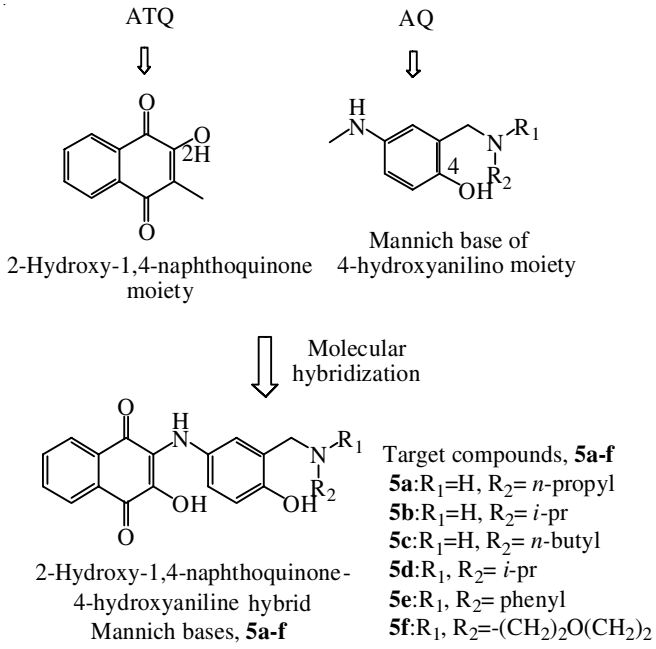


Fig. 2. Design of novel hybrid compounds

In the present study, some new 2-hydroxy-1,4-naphthoquinone-4-hydroxyaniline hybrid Mannich Bases were designed, synthesized and screened for *in vitro* antimalarial activity. The synthesized compounds were also evaluated *in silico* for molecular properties assessment and drug-likeness prediction with an aim to study their drug-like characteristics based on Lipinski's rule of five with additional parameters.

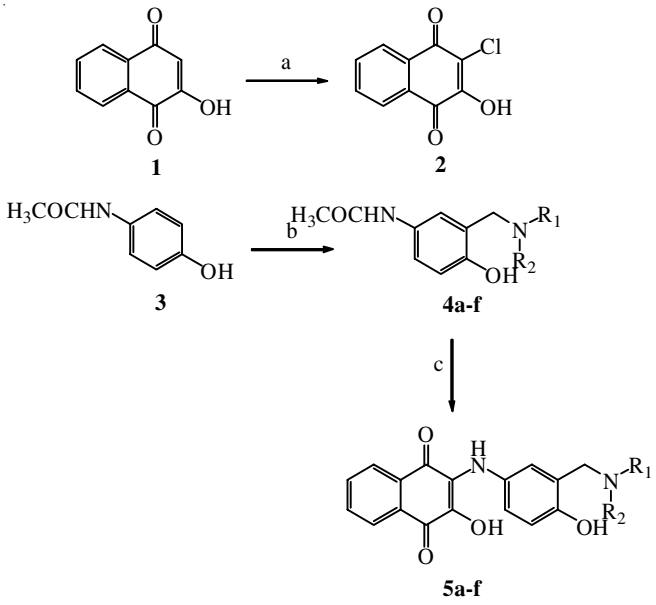
Molecular properties are fundamental structural properties which determine the physicochemical (solubility, permeability) and biochemical (metabolic stability, transport property, protein/tissue affinity) properties, which in turn ultimately determine molecule's pharmacokinetics (bioavailability, half life), toxicity and pharmacodynamics (receptor affinity and efficacy) in biological systems [15]. Molecular properties are therefore required to be assessed in order to evaluate the drug-like characteristics of a particular molecule based on Lipinski's rule of five.

EXPERIMENTAL

All of the chemicals were procured commercially from Sigma-Aldrich Corporation (USA), Merck Specialists Pvt. Ltd. (Germany), HiMedia Lab. Pvt. Ltd. (Germany) or Spectrochem Pvt. Ltd. (India) and were used without further purification, unless otherwise stated. The solvents and reagents used in the antimalarial study were of analytical grade. The progress of reactions was monitored by the silica gel-G thin-layer chromatography (TLC) and the spots were visualized by iodine vapours. Melting points (m.p.) were measured in open capillaries on an electrically heated melting point apparatus. UV-visible spectra were recorded on Shimadzu UV-1700 UV-visible spectrophotometer and the wavelength of maximum absorption (λ_{max}, nm) is reported. Infrared (IR) spectra were obtained on a Bruker Alpha FT-IR spectrometer using KBR disc and are reported in terms of frequency of absorption (ν, cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AD II 400 FT-NMR spectrometer at 400 and 100 MHz, respec-

tively with tetramethylsilane (TMS) as an internal standard (δ 0.00 ppm) and $CDCl_3$ as a solvent. Chemical shift (δ) values were expressed in parts per million (ppm) relative to TMS (δ 0.00 ppm). ^{1}HNMR data are assigned in order: peak multiplicity (b, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons (numerical integral value), coupling constants (*J* value) in hertz. Mass spectra were obtained with the LC-MS Water 4000 ZQ instrument using atmospheric pressure ionization (API-ES) in the range of *m/z* 150-1400 both in the negative and positive ion modes. The *m/z* values of the most intense quasimolecular ion $[M+H]^+$ peak, with relative intensities in parentheses are stated.

General method of synthesis: The target compounds were synthesized as Mannich bases of 2-hydroxy-1,4-naphthoquinone (lawsone)-4-hydroxyaniline conjugate. 2-hydroxy-1,4-naphthoquinone (**1**) obtained commercially was first chlorinated by thionyl chloride to give 3-chloro-2-hydroxy-1,4-naphthoquinone (**2**) which was later fused with different Mannich products of 4-aminophenol to afford hybrid compounds **5a-f**. The Mannich reaction of commercially available 4-acetamidophenol (**3**) and subsequent hydrolysis of the amide function led to the formation of substituted Mannich bases, **4a-f** of 4-aminophenol/4-hydroxyaniline. The general procedure [11] of synthesis of target compounds is depicted in **Scheme-I**.



Scheme-I: Synthesis of target compounds (**5a-f**). Reagents and conditions: (a) $SOCl_2$, pyridine, 70 °C, 1 h; (b) primary/secondary amine, Aq. CH_2O, EtOH, reflux, 24 h; (c) (i) 2O % HCl, EtOH, reflux, 6 h; (ii) 2, EtOH, reflux 12 h

To a mixture of 2-hydroxy-1,4-naphthoquinone (7 g, 40 mM) and pyridine (3.2 g, 3.2 mL, 40 mM) thionyl chloride (6 mL) was added drop wise and the resulting solution was heated under reflux at 70 °C for about 1 h. The solvent was removed under vacuum and the product thus separated was collected.

A mixture of 4-acetamidophenol (6 g, 39.69 mM), appropriate primary/secondary amine (39.69 mM) and aqueous formaldehyde (2.46 mL) in ethanol (28.29 mL) was heated under reflux for 24 h. The solvent was then removed under reduced pressure and the residue was dissolved in dichloromethane. The organic solution was treated with dilute hydrochloric acid, basified to pH 9-10 and then again extracted with dichloromethane. The combined extract was washed with water and the separated product was collected.

A solution of N-(4-hydroxy-3-[(substituted amino)-methyl]phenyl)acetamide (17 mM) in hydrochloric acid (20 %, 27.3 mL) was heated under reflux for 6 h. The solvent was then removed under reduced pressure and the resulting residue was co-evaporated with ethanol. The residue was dissolved in ethanol (20 mL), 3-chloro-2-hydroxy-1,4-naphthoquinone (3.8 g, 18 mM) was added into it and the mixture was heated under reflux for 12 h. The solution was concentrated under reduced pressure to give a viscous mass which was subsequently poured into ice-cold ammonium hydroxide. The sticky solid thus separated was dissolved in dichloromethane and was extracted with alkaline solution. The organic solution was washed with water, dried with anhydrous sodium sulphate and then evaporated to dryness under reduced pressure to give the crude product. The product was recrystallized from dichloromethane.

***In vitro* Antimalarial activity screening [3,12,16,17]:** All the synthesized compounds, **5a-f** were screened for *in vitro* antimalarial activity against a chloroquine-sensitive strain of *P. falciparum* (RKL-2) and additionally, two compounds, **5a** and **5f** were also screened for activity against chloroquine-resistant strain of *P. falciparum* (RKL-9). The antimalarial activity screening was carried out by Giemsa stained slide method in the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

Continuous culture of *P. falciparum* strain was maintained *in vitro* in O^+ human red blood cells diluted to 6 % haematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, 1 % D-glucose, 0.23 % sodium bicarbonate, gentamycin (40 mg/mL), amphotericin-B (0.25 mg/mL) and 10 % human AB^+ serum. Incubations were done at 37 °C and 5 % CO_2 level in a modular incubator. D-sorbitol synchronized 1 % ring stage parasitaemia in 3 % haematocrit was used for antimalarial assays using 96 well microtitre plate. A stock solution of 5 mg/mL of the test compound was prepared in DMSO and subsequent dilutions were made with incomplete RPMI in duplicate. All the test compounds were assayed at a fixed dose of 1 mg/mL. Each test well of the microtitre plate contained 20 µL of the compound and 180 µL of 1 % ring stage parasitaemia in 3 % haematocrit. In addition, drug free negative control to assess the parasite growth and chloroquine diphosphate (at 0.1 mg/mL dose) as positive control to assess the integrity of the assay were also maintained in duplicate in the microtitre plate. After 40 h of incubation the smears were prepared from each well, stained with 3 % Giemsa and scanned under light microscope to ascertain percentage dead rings and trophozoites by examining a minimum of 400 asexual parasites.

Test results were compared with the standard result of chloroquine. Each test compound was assayed in two replicates and counted against 400 asexual parasites (% dead rings + trophozoites) per replicate. The percentage of inhibition of parasite growth was obtained as mean of duplicate studies. The minimum inhibitory concentration (MIC) and IC_{50} values (in µg/mL) were calculated using the NonLin V1.1 software.

Molecular properties and drug-likeness prediction: Drug-like properties of the synthesized compounds, **5a-f** was studied *in silico* using web based Molsoft (molsoft.com/molprop/) and Molinispiration Cheminformatics (www.molinspiration.com) academic softwares. The following molecular descriptors were calculated for drug-likeness prediction: Molecular weight, water solubility (log S), octanol-water partition coefficient (log P), hydrogen bond acceptor and donor count, polar surface area (PSA), rotable bonds. The drug-likeness score were also calculated for all the synthesized compounds.

RESULTS AND DISCUSSION

In the present study, a new series of 2-hydroxy-1,4-naphthoquinone-4-hydroxyaniline hybrid Mannich bases were synthesized and evaluated for *in vitro* antimalarial activity. Several acyclic and cyclic primary/secondary amines were used for different Mannich substitutions on a conjugated framework of 2-hydroxy-1,4-naphthoquinone (lawsone) and 4-hydroxyaniline scaffolds. The target compounds are thus the Mannich bases of hybrid skeleton made up of two distinct pharmacophoric moieties from two potent antimalarial drugs, atovaquone and amodiaquine, respectively. The physicochemical details of the synthesized compounds are given in Table-1. The purity of the synthesized compounds was ascertained by melting point determinations and silica gel G TLC. The R_f values of the synthesized compounds are reported herein. All the compounds were obtained in good yields with high purity. The structural assignments of the synthesized compounds were made on the basis of UV, FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The spectral data of **5a-f** are depicted below:

TABLE-1
PHYSICO-CHEMICAL DATA OF
SYNTHESIZED COMPOUNDS, **5a-f**

Comp.	m.f.	m.w.	Yield (%)	m.p. (°C)	R_f value*
5a	$C_{20}H_{20}N_2O_4$	352.14	57.54	57-59	0.67
5b	$C_{20}H_{20}N_2O_4$	352.14	82.54	71-74	0.44
5c	$C_{21}H_{22}N_2O_4$	366.16	70.76	81-84	0.49
5d	$C_{23}H_{26}N_2O_4$	394.19	89.86	149-152	0.97
5e	$C_{29}H_{22}N_2O_4$	462.16	58.56	47-49	0.87
5f	$C_{21}H_{20}N_2O_5$	380.14	68.67	141-143	0.21

*Solvent system: Dichloromethane/MeOH-5:5

2-Hydroxy-3-(4-hydroxy-3[(propylamino)methyl]-phenylamino)-2,3-dihydronaphthalene-1,4-dione (5a): UV spectrum (dichloromethane), λ_{max} (nm): 250.0; IR (KBr, ν_{max}, cm^{-1}): 3526, 3454 (2 O-H), 3345, 3327 (2 N-H), 3015, 3008 (C-H aryl), 2962, 2920, 2866, 2824 (C-H, CH_3 & CH_2), 1678, 1630 (2 C=O), 1620, 1592, 1543, 1490 (C=C aryl), 1363, 1350 (C-N), 1210, 1175 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.93 (bs, 1H, alcoholic OH), 8.02-7.96 (m, 4H, Ar-H), 6.64 (d, 1H, *J* = 7.86 Hz, Ar-H), 6.57 (d, 1H, *J* = 7.68 Hz, Ar-H), 6.42 (s, 1H, Ar-H); 6.36 (bs, 1H, phenolic OH), 5.63 (s, 1H, NH-aryl), 3.67 (s, 2H, CH_2-NH), 3.05 (s, 1H, NH-Alkyl), 2.22 (t, 2H, *J* = 7.62 Hz, NH-CH_2CH_2), 1.46 (m, 2H, $CH_2CH_2CH_3$), 1.13 (t, 3H, *J* = 7.67 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 184.46, 178.32 (2 C=O), 152.32, 126.26 (2 C-OH), 138.50, 132.23, 130.24, 128.26, 123.26, 118.10 (aromatic CH), 115.39 (olefinic C=C, CO-C=C-CO), 52.20, 49.27, 32.65, 12.21 (aliphatic CH_2 & CH_3); MS (API), *m/z* (%): 353.18 (100), $[M+H]^+$.

2-Hydroxy-3-(4-hydroxy-3-[(isopropylamino)methyl]-phenylamino)-2,3-dihydronaphthalene-1,4-dione (5b): UV spectrum (dichloromethane), λ_{max} (nm): 243.5; IR (KBr, ν_{max}, cm^{-1}): 3520, 3452 (2 O-H), 3344, 3329 (2 N-H), 3015, 3010 (C-H aryl), 2962, 2922, 2868, 2826 (C-H, CH_3 & CH_2), 1677, 1635 (2 C=O), 1620, 1590, 1544, 1488 (C=C aryl), 1364, 1352 (C-N), 1211, 1178 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.90 (bs, 1H, alcoholic OH), 8.06-7.98 (m, 4H, Ar-H), 6.62 (d, 1H, *J* = 7.88 Hz, Ar-H), 6.56 (d, 1H, *J* = 7.58 Hz, Ar-H), 6.43 (s, 1H, Ar-H), 6.37 (bs, 1H, phenolic OH), 5.64 (s, 1H, NH-aryl), 3.66 (s, 2H, CH_2-NH), 3.07 (s, 1H, NH-alkyl), 2.29 (m, 1H, NH-$CH(CH_3)_2$), 1.08 (d, 6H, *J* = 7.88 Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 184.86, 178.00 (2 C=O), 154.67, 128.02 (2 C-OH), 138.72, 133.93, 130.25, 128.20, 124.52, 118.00 (aromatic CH), 115.39 (olefinic C=C, CO-C=C-CO), 48.66, 46.28, 12.80 (aliphatic CH_2, CH & CH_3); MS (API), *m/z* (%): 353.70 (100), $[M+H]^+$.

2-Hydroxy-3-(3-hydroxy-4-[(butylamino)methyl]-phenylamino)-2,3-dihydronaphthalene-1,4-dione (5c): UV spectrum (dichloromethane), λ_{max} (nm): 247.5; IR (KBr, ν_{max}, cm^{-1}): 3530, 3460 (2 O-H), 3355, 3328 (2 N-H), 3012, 3002 (C-H aryl), 2966, 2927, 2865, 2830 (C-H, CH_3 & CH_2), 1674, 1632 (2 C=O), 1612, 1583, 1556, 1488 (C=C aryl), 1376, 1358 (C-N), 1212, 1180 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.90 (bs, 1H, alcoholic OH), 8.04-7.98 (m, 4H, Ar-H), 6.63 (d, 1H, *J* = 7.68 Hz, Ar-H), 6.54 (d, 1H, *J* = 7.82 Hz, Ar-H), 6.42 (s, 1H, Ar-H), 6.38 (bs, 1H, phenolic OH), 5.64 (s, 1H, NH-aryl), 3.63 (s, 2H, CH_2-NH), 3.08 (s, 1H, NH-alkyl), 2.32 (t, 2H, *J* = 7.88 Hz, NH-CH_2CH_2), 2.02 (m, 2H, $CH_2CH_2CH_2$), 1.48 (m, 2H, $CH_2CH_2CH_3$), 1.09 (t, 3H, *J* = 7.64 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 186.40, 178.46 (2 C=O), 155.00, 127.20 (2 C-OH), 138.52, 132.00, 131.58, 128.36, 124.22, 118.36 (aromatic CH), 115.30 (olefinic C=C, CO-C=C-CO), 52.68, 46.20, 38.29, 33.62, 30.27, 12.10 (aliphatic CH_2 & CH_3); MS (API), *m/z* (%): 367.61 (100), $[M+H]^+$.

2-(4-[(Diisopropylamino)methyl]-3-hydroxyphenylamino)-3-hydroxy-2,3-dihydronaphthalene-1,4-dione (5d): UV spectrum (dichloromethane), λ_{max} (nm): 250.0; IR (KBr, ν_{max}, cm^{-1}): 3540, 3452 (2 O-H), 3350, 3335 (2 N-H), 3018, 3000 (C-H aryl), 2964, 2922, 2860, 2826 (C-H, CH_3 & CH_2), 1677, 1640 (2 C=O), 1632, 1586, 1563, 1498 (C=C aryl), 1364, 1352 (C-N), 1212, 1165 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.87 (bs, 1H, alcoholic OH), 8.02-7.96 (m, 4H, Ar-H), 6.66 (d, 1H, *J* = 7.52 Hz, Ar-H), 6.54 (d, 1H, *J* = 7.48 Hz, Ar-H), 6.43 (s, 1H, Ar-H), 6.37 (bs, 1H, phenolic OH), 5.65 (s, 1H, NH-aryl), 3.66 (s, 2H, CH_2-N), 2.28 (m, 1H, NH-$CH(CH_3)_2$), 1.06 (d, 6H, *J* = 7.45 Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 186.04, 178.54 (2 C=O), 155.54, 128.32 (2 C-OH), 138.26, 132.39, 131.60, 128.00, 124.42, 118.00 (aromatic CH), 115.26 (olefinic C=C, CO-C=C-CO), 48.40, 46.72, 12.20 (aliphatic CH, CH_2 & CH_3); MS (API), *m/z* (%): 395.26 (100), $[M+H]^+$.

2-(4-[(Diphenylamino)methyl]-3-hydroxyphenylamino)-3-hydroxy-2,3-dihydronaphthalene-1,4-dione (5e): UV spectrum (dichloromethane), λ_{max} (nm): 242.0; IR (KBr, ν_{max}, cm^{-1}): 3530, 3450 (2 O-H), 3376, 3330 (2 N-H), 3016,

3000 (C-H aryl), 2966, 2928, 2867, 2825 (C-H, CH_3 & CH_2), 1680, 1634 (2 C=O), 1610, 1595, 1544, 1492 (C=C aryl), 138, 1352 (C-N), 1212, 1180 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.95 (bs, 1H, alcoholic OH), 8.05-7.98 (m, 4H, Ar-H), 7.18-7.09 (m, 5H, Ar-H),, 6.92-6.85 (m, 5H, Ar-H), 6.63 (d, 1H, *J* = 7.54 Hz, Ar-H), 6.58 (d, 1H, *J* = 7.64 Hz, Ar-H), 6.52 (s, 1H, Ar-H), 6.47 (bs, 1H, phenolic OH), 5.52 (s, 1H, NH-Aryl), 3.58 (s, 2H, CH_2-N); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 186.24, 176.53 (2 C=O), 156.68, 125.20 (2 C-OH), 146.23, 138.26, 136.34, 132.53, 130.02, 128.61, 129.02, 124.26, 119.26, 118.10 (aromatic CH), 116.32 (olefinic C=C, CO-C=C-CO), 48.63 (CH_2); MS (API), *m/z* (%): 3467.20 (100), $[M+H]^+$.

2-Hydroxy-3[4-hydroxy-3-(morpholinomethyl)cyclohexylamino]-2,3-dihydronaphthalene-1,4-dione (5f): UV spectrum (dichloromethane), λ_{max} (nm): 248.5; IR (KBr, ν_{max}, cm^{-1}): 3522, 3455 (2 O-H), 3365, 3338 (2 N-H), 3018, 3000 (C-H aryl), 2968, 2936, 2864, 2837 (C-H, CH_3 & CH_2), 1677, 1645 (2 C=O), 1622, 1590, 1544, 1460 (C=C aryl), 1364, 1362 (C-N), 1226, 1180 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.89 (bs, 1H, alcoholic OH), 8.04-7.96 (m, 4H, Ar-H), 6.96 (d, 1H, *J* = 7.40 Hz, Ar-H), 6.72 (d, 1H, *J* = 7.38 Hz, Ar-H), 6.55 (s, 1H, Ar-H); 6.34 (bs, 1H, phenolic OH), 5.60 (s, 1H, NH-aryl), 3.53 (s, 2H, CH_2-N), 3.46 (t, 2H, *J* = 7.32 Hz, morpholinyl CH_2), 2.39 (t, 2H, *J* = 7.46 Hz, morpholinyl CH_2); ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 185.02, 178.36 (2 C=O), 158.46, 128.28 (2 C-OH), 138.20, 134.24, 130.60, 128.35, 124.40, 118.00 (aromatic CH), 115.04 (olefinic C=C), 65.32 (morpholinyl CH_2), 52.06 (morpholinyl CH_2), 49.44 (CH_2); MS (API), *m/z* (%): 381.29 (100), $[M+H]^+$.

The spectral data depicted above are in close agreement with the structures of the synthesized compounds, **5a-f**. All the compounds in dichloromethane exhibited characteristic absorption maxima (λ_{max} in the range of 240-250 nm) indicating the presence of chromophoric 2-hydroxy-1,4-naphthoquinone system embedded aminoaryl moiety containing auxochromes like phenolic hydroxyl group and alkyl/hetero ring substituted amino methyl chain. The infrared spectral data showed characteristic absorption bands for hydroxyl groups (O-H str., broad peaks in the frequency range of 3540-3520 cm^{-1} and 3460-3450 cm^{-1} for phenolic and alcoholic, respectively), 2° amino moieties (Aryl N-H str. and alkyl N-H str., peaks of medium intensity in the range of 3376-3328 cm^{-1}), two cyclic carbonyl functions (C=O str., sharp peaks around 1670 cm^{-1} and 1640-1630 cm^{-1}) and methylene group (C-H str., about 2900 cm^{-1} and about 2800 cm^{-1}) [18], which ascertains the anticipated structure of the synthesized compounds. ^{1}H NMR spectra displayed signals (chemical shift values, δ) for different structural protons of aryl components of conjugated naphthoquinone-aniline framework along with OH (broad singlet(s) at δ 9.9 and δ 6.3-6.4 for alcoholic and phenolic, respectively), NH (sharp singlet(s) around δ 5.6 for 2° aryl amino and δ 3.05, 3.07, 3.08 for 2° alkyl amino, **5a-c**) and methylene protons (-CH_2NH/CH_2N, approximate δ values between 3.5-3.6) [20] of the synthesized compounds. The ^{13}C resonance data revealed the presence of different carbon atoms such as aryl-C, C=O, CH_2, CH_3, *etc*. in the structural scaffold of 1,4-napthoquinoe nucleus and aminoaryl residue along with substituted amino methyl Mannich side chain. The mass spectra of **5a-f** exhibited prominent molecular ion peaks, $[M+H]^+$ which are in accordance with the anticipated mass corresponding to their respective molecular formula.

Antimalarial activity: The *in vitro* antimalarial activity data is depicted in Table-2. The IC_{50} (in μg/mL) represents the concentration of compound that inhibits the growth of malaria parasite by 50 %. Results clearly reveal that all the synthesized compounds, **5a-f** showed activity against chloroquine-sensitive *P. falciparum* (RKL-2) strain at the tested dose which, however, was considerably less than that of the standard reference drug, chloroquine. Among the synthesized compounds, compounds substituted with *n*-propyl (**5a**, IC_{50} 0.453 μg/mL) and morpholinyl (**5f**, IC_{50} 0.391 μg/mL) moieties showed comparatively better activity against the sensitive strain of *P. falciparum* than rest of the compounds having substitutions like isopropyl (**5b**, IC_{50} 0.639 μg/mL), *n*-butyl (**5c**, IC_{50} 1.263 μg/mL), diisopropyl (**5d**, IC_{50} 0.613 μg/mL) and biphenyl (**5e**, IC_{50} 0.911 μg/mL) moieties. The IC_{50} value for chloroquine in sensitive strain of *P. falciparum* was found 0.0391 μg/mL. It is apparent that compounds with smaller alkyl substitutions such as *n*-propyl and isopropyl groups and with saturated heterocycle such as morpholinyl moiety possess superior antimalarial effectiveness in comparison to other compounds having bulky alkyl or aryl substitutions such as *n*-butyl group and phenyl rings.

TABLE-2
In vitro ANTIMALARIAL ACTIVITY DATA[#]

Comp. code[$]	MIC (μg/mL)[*]	IC_{50} (μg/mL)[*]
5a	3.12 (> 25[**])	0.453 (2.921[**])
5b	12.5	0.639
5c	25	1.263
5d	12.5	0.613
5e	> 25	0.911
5f	1.56 (> 25[**])	0.391 (0.993[**])
Chloroquine[$$]	3.125 (> 25[**])	0.0391 (0.299[**])

[#]Data are presented as mean of duplicate observations.
[*]CQ-sensitive *P. falciparum* (RKL-2) strain.
[**]CQ-resistant *P. falciparum* (RKL-9) strain.
[$]Test dose: 1 mg/mL.
[$$]Standard dose: 0.1 mg/mL.

Compound **5a** and compound **5f** were further tested for antimalarial activity against the resistant strain of *P. falciparum* (RKL-9) and were found less active (IC_{50} 2.921 and 0.993 μg/mL respectively) than that of the standard chloroquine (IC_{50} 0.299 μg/mL). However, compound **5f** possesses higher antimalarial effectiveness than compound **5a** in resistant *P. falciparum* strain. It has been reported [11] that replacement of the diethyl functional moiety at the Mannich side chain of 4-aminoquinoline phenol conjugates with the metabolically stable alkyl grouping and heterocyclic ring as in morpholinyl modifications lead to a substantial increase in the antimalarial activity of the compound.

Drug-likeness assessment: Drug-like properties are very important in assessing that whether a particular molecule satisfies all desirable properties of the known drugs or not. In general, drug-like properties include both physicochemically significant descriptors and pharmacokinetically relevant

properties. Drug-likeness prediction evaluates the acceptability of derivatives as drug molecules based on Lipinski's rule of five [19].

The results of predicted drug-like properties and drug-likeness score of the synthesized compounds, **5a-f** is presented in Table-3. All the compounds possess good drug-like properties based on Lipinski's rule of five with additional parameters such as log S and polar surface area. Poor absorption or permeation of a ligand is more likely when there are molecular weight > 500, log $P_{o/w}$ > 5, number of HBA > 10, number of HBD > 5 and number of Rot B > 5 [15]. Hydrophobicity, membrane permeability and bioavailability are always associated with molecular weight, log P, log S and hydrogen bond acceptor and donor count, *etc*. Molecules violating more than one of these rules may have problems with bioavailability. Sufficient water solubility is important for optimal bioavailability of drugs. Number of rotable bonds is also important for molecular conformational studies (*i.e.*, stereoselectivity of drug molecules) required for optimal binding with the receptor. Molecular polar surface area is a useful parameter for drug transport properties [20].

The values of the calculated drug-like properties are in acceptable range for all the compounds except compound **5e** which violated one rule because of having log P value more than 5 (5.69). Compounds **5a-e** possess less than or equal to 4 hydrogen bond donors and have no more than 6 hydrogen bond acceptors. The octanol-water partition coefficient (log P) is less than 5 for all these compounds. None of their molecular weights exceeds 500 daltons. The number of rotable bonds and polar surface area are in permissible range for all of the compounds. Furthermore, the drug-likeness score of the compounds, **5a-5f** ranges from 0.18-0.95. Higher drug-likeness scores were found 0.95 and 0.91 for compounds **5b and 5f** with molecular weights of 352.14 and 380.14, log P values of 2.48 and 2.01, log S values of -5.44 and -3.90, polar surface area values of 77.98 and 78.82, 5 and 6 hydrogen bond acceptors (HBA), 4 and 3 hydrogen bond donors (HBD) and 5 and 4 rotable bonds, respectively. The results of drug-likeness studies strongly suggest that newly synthesized compounds have drug-likeness behaviour favourable for membrane permeability, transport and bioavailability and also interaction with receptor. Assessment of drug-likeness score implies that the suitability of derivatives as drug-like molecules. Compounds having zero or negative value should not be considered as drug-like molecule.

Since all the studied compounds showed favourable drug-like properties a reasonable correlation can be drawn between the calculated drug-like properties and *in vitro* antimalarial activity profile of the compounds **5a-5f**. log $P_{o/w}$ is a direct indicator of lipophilicity of drug substances. Higher the value of log P, better the membrane permeability. It is essential for a molecule to have sufficient lipophilicity for optimal bioavailability and drug action. Hydrogen bond acceptor and donor groups are of paramount importance in this regard. Polar surface area is closely related to the hydrogen bonding potential required for the bio-efficacy of a drug molecule. Practically, a compound with all drug-like properties in the desired range appears to exhibit high levels of therapeutic potency. Good drug-like properties and activity are complementary and hence balanced attention to both properties and bioactivity could suitably transform a ligand to a good drug lead [15].

Higher antimalarial activity of the compound **5f** might be because of increased lipophilicity of the molecule. Polar properties such as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) groups, polar surface area, *etc*. which contribute transport property to the drug molecule are responsible as well. Having desired lipophilicity compound **5f** (log P 2.01) readily penetrated parasite's cell membrane that led to attain an intracellular concentration which was favourable for antimalarial action. A good correlation also exists between other drug-like properties (HBA/HBD groups, polar surface area, rotable bonds) and antimalarial activity of **5f**. Drug-likeness score of the compound (**5f**, 0.91) is in support of the above consideration.

Finally, it can be concluded that 4-hydroxy-1,4-naphthoquinone (lawsone) coupled with 4-hydroxyaniline moiety is attributed to have potential as a new hybrid antimalarial scaffold. Different Mannich substitutions have contributing effects towards improving the antimalarial efficacy of the basic nuclear system. Some degree of variations in activity among synthesized hybrid compounds might be because of different alkyl/heterocyclic Mannich substitution pattern. From earlier studies [11], it is apparent that the presence of a 4-arylamino moiety and an aryl hydroxyl function appear to be important for activity both in terms of resistant preventing activity and antiparasitic activity. It is noteworthy to mention here that the position of amino and hydroxyl functions in the aryl residue (as in amodiaquine) would render molecule toxic (as it forms toxic quinoneimine metabolites) [13,14]. However, because of having antimalarial effectiveness newer hybrid Mannich bases can be used as such as lead molecules for further structural modification/optimization to achieve an array of similar compounds/analogous with better activity profile. From another point of view, rational design of some different structural

TABLE-3
RESULTS OF PREDICTED DRUG-LIKENESS PROPERTIES

Comp.	m.w.	log P[a]	log S[b]	HBA[c]	HBD[d]	Rot B[e]	PSA[f] (A^2)	Violation of rule of 5	Drug-likeness score
5a	352.14	2.65	-5.29	5	4	6	79.06	0	0.85
5b	352.14	2.48	-5.44	5	4	5	77.98	0	0.95
5c	366.16	3.13	-5.54	5	4	7	79.06	0	0.65
5d	394.19	3.76	-5.68	5	3	6	70.55	0	0.79
5e	462.16	5.69	-7.62	4	3	6	69.34	1	0.18
5f	380.14	2.01	-3.90	6	3	4	78.82	0	0.91

[a]Octanol/water partition coefficient, calculated lipophilicity; [b]Water solubility in log (mol/L); [c]Number of hydrogen bond acceptor (HBA); [d]Number of hydrogen bond donor (HBD); [f]Polar surface area.

derivatives as potent antimalarial agents can be made on the basis of conjugated 2-hydroxy-1,4-naphthoquinone-4-hydroxyaniline hybrid scaffold. Metabolic and toxicity studies are important to be carried out for optimizing those structural congeners. To overcome the problem of toxicity, the 3-Mannich side chain can be interchanged with the 4-OH function which does not generate toxic quinoneimine metabolites (as in isoquine) [11].

Conclusion

The present work investigates the synthesis, *in vitro* antimalarial activity and drug-likeness prediction of some newer Mannich bases of 2-hydroxy-1,4-naphthoquinone-4-hydroxyaniline hybrid. The synthesis and antimalarial activity of these hybrid molecules have not been reported earlier. The structural assignments of the new compounds were made on the basis of IR spectroscopy, NMR spectroscopy and Mass spectrometric analyses. All the synthesized compounds exhibited some degree of *in vitro* antimalarial activity against the chloroquine-sensitive strain (RKL-2) of *P. falciparum* at the tested dose, which, however, was considerably less than that of standard drug, chloroquine. Two of the synthesized compounds with propyl and morpholinyl substitutions showed good activity, whereas other compounds showed moderate activity. Since all the compounds exhibited favourable drug-likeness behaviour a reasonable correlation therefore appears to exist between their drug-like properties and antimalarial activity profile. On the basis of our present findings, future work can be directed towards the rational design of such more hybrid analogous in structurally diverse series based on the nuclear scaffold of lawsone (4-hydroxy-1,4-naphthoquinone) by replacement and/or modification of existing moieties/groups with more metabolically stable moieties at the Mannich side chain. This strategy would possibly lead to the development of new potent yet safe antimalarial agents.

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