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Synthesis and *In-Vivo* Pharmacological Evaluation of Some Novel 4(3H)-Quinazolinone Derivatives as Potential Anti-malarial Agents

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Abstract

In this work six 3-aryl-2-(substituted styryl)-4(3H)-quinazolinones derivatives were synthesized by the reaction of 3-aryl-2-methyl-4(3H)-quinazolinone (intermediate products)with different substituted aromatic aldehydes. Three intermediate products were synthesized by reacting 2-methyl-3,1-benzoxazin-4-one, which was initially prepared by cyclizing anthranilic acid using acetic anhydride, with three aromatic amines. Their structures were confirmed using IR, ^{1}HNMR, ^{13}CNMR spectroscopic methods and elemental microanalyses. The synthesized compounds were evaluated for their in vivo antimalarial activity against *P. berghei*. Four of the synthesized compounds (IIIc, IVa, IVb and IVf) exhibited activity against the parasite. Among these compound IVa was found to be the most active compound. Results of acute toxicity study showed that oral administration of the synthesized compounds in single doses (100, 250 and 500 mg/kg) had no adverse effects, indicating that the compounds have high safety margin and their LD_{50} is higher than 500 mg/kg.In general this study indicates that 4(3H)-quinazolinones derivatives are potential sources of lead compounds for ant malarial drugs.

Keywords: 4(*3H*)-quinazolinones derivatives; *In vivo; P. berghei;* Anti-malarial

Introduction

Malaria is one of the oldest recorded diseases in the world. Ancient Chinese and Sanskrit medical texts described its symptoms and Hippocrates referred to the disease in the 4th Century BC [1]. It is estimated to account for 300 million to 500 million illnesses and nearly 1 million deaths each year [2]. Malaria is a protozoal disease caused by parasites of the genus *Plasmodium* [3]. Four identified species of this parasite exist, which cause different types of human malaria, namely; *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malariae* [4,5]. Ethiopia had approximately 6% of malaria cases in the African Region in 2006 [6]. Almost 75% of the country's land is malarious and an estimated 51 million (68%) of the people.

In the past 30 years, only one synthetic antimalarial drug (mefloquine) has been discovered. The other drug discovered during this period, artemisinin, is a natural product, whose medicinal properties were known for more than 2,000 years [7]. The sudden and dramatic resurgence of malaria in many countries have made synthetic efforts toward new antimalarial drugs very important [8]. Recently some synthetic compounds are reported to have potent antimalarial activity against different *Plasmodium* species.

Clinical trials conducted with fosmidomycin, chalcone analogue, naphtha quinone, acylation of the hydroxy moiety of atovaquone derivatives and aryl sulfonyl acridinyl derivatives were reported to have outstanding antimalarial activity [7,9-12]. Quinazolinones are versatile nitrogen heterocyclic compounds, diplaying wide applications including anticonvulsant, sedative, tranquilizer, analgesic, antimicrobial, anesthetic, anticancer, antihypertensive, anti-inflammatory, antimalarial, diuretic and muscle relaxant properties [13-22]. Increased efforts in antimalarial drug discovery are urgent to develop safe and affordable new drugs to counter the spread of malaria parasites that are resistant to existing agents. Furthermore, quinazolinones substituted at 2 and 3- position play a pivotal role in the antimalarial activity [13].

Several bio-active natural products such as febrifugine and isofebrifugine contain quinazolinone moieties with potential antimalarial activity were also reported [11,12]. Therefore, 2,3-disubstituted-4(*3H*)-quinazolinones, are point of interest to seek for new drugs that act against the malarial pathogen in order to combat and reduce its tremendous prevalence. Hence, in this work compounds containing 4(*3H*)-quinazolinone moiety were designed to study their antimalarial activities. The simple synthesis and anti malarial results of these newly synthesized compounds are reported.

Experimental

General

Melting points were determined in open capillaries using Buchi (B-540) melting point apparatus and are uncorrected. IR spectra were recorded in nujol SHIMADZU8400SP FT-IR spectrophotometer,^{1}HNMR and ^{13}CNMR spectra in $CDCl_3/CCl_4$ in Bruker Avance DMX-400 FT-NMR spectrometer using TMS as internal reference (chemical shifts in δ, ppm). Elemental microanalyses were performed on Perkin Elmer 2400 elemental analyzer. Elemental (C, H, N) analysis indicated that calculated and observed values were within ± 0.4 of theoretical values. The purity of compounds was checked by thin layer chromatography on silica gel plate of 0.25 mm thickness using benzene: methanol (9:1) as a solvent system. Iodine chamber was used as a developing chamber. All the reagents used were AR grade.

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General procedure for the preparation of 3-aryl-2-methyl-4(3H)-quinazolinones (III a-c)

A mixture of acetanthranil **II**, (0.1 mol) and an equimolar amount of the appropriate aromatic amine was heated under reflux for 5-7 hrs. The dark sticky mass formed was cooled and recrystallized from ethanol.

2-Methyl-3-anilino-3H-quinazolin-4-one (IIIc)

Yield 69.6%; mp 187-189°C; IR (Nujol) (cm^{-1}): 3264 (NH); 1675 (C=O) ; 1649 (C=N). ^{1}HNMR ($CDCl_3$/CCl_4) δ ppm: 2.53 (*s*, *3H*, CH_3), 6 .56 (*d*, 2H, anilino $C_{2,6}$ H), 6.8 (*t*, 1H, anilino C_4 H), 7.1 (*t*, 2H, anilino $C_{3,5}$ H), 7.35 (*t*, 1H, quinazolinone C_6 H), 7.56 (*d*, 1H, quinazolinone C_8 H), 7.67 (*t*, 1H, quinazolinone C_7 H) and 8.06 (*d*, 1H, quinazolinone C_5 H). ^{13}CNMR ($CDCl_3$/CCl_4) δ ppm: 21.09 (1C, CH_3), 113.13 (2C, anilino $C_{2,6}$), 120.83, (1C, anilino C_4), 121.91 (1C, quinazolinone C_{4a}), 126.51 (1 C, quinazolinone C_6), 126.59 (1C, quinazolinone C_8), 126.65 (1C, quinazolinone C_5), 129.27 (2C, anilino $C_{3,5}$), 134.79 (1 C, quinazolinone C_7), 145.78 (1 C, quinazolinone C_{8a}), 146.61 (1C, anilino C_1), 157.68 (1 C, 4(*3H*)-quinazolinone C_2) and 161.12(1C, $\underline{C}$=O).Anal. Calcd. for$C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58. Found: C, 68.47; H, 4.47; N, 14.42.

General procedure for the preparation of 3-aryl-2-(substituted styryl)-4(3H)-quinazolinones (IVa-f)

A mixture of 3-aryl-2-methyl-4(*3H*)-quinazolinone **IIIa-c,** (10 mmol) and an equimolar amount of the appropriate aromatic aldehyde was allowed to react in the presence of fused sodium acetate by heating under reflux for 10-12 hrs. The solid products formed were filtered, washed with ethanol, dried and recrystallized from ethanol.

4-[(1*E*)-2-(3,4-dihydro-4-oxo-3-*p*-tolylquinazolin-2-yl) vinyl]-2-methoxy phenylacetate (IVa)

Yield 53%; mp 206-208°C; IR (Nujol) (cm^{-1}): 1760 (C=O); 1670 (C=N); 1655 (C=O); 1220(C-O); 1119 (C-O); ^{1}HNMR ($CDCl_3$/$CDCl_4$) δppm: 2.3 (*s*, *3H*, *p*-tolyl $C\underline{H}_3$), 2.5 (*s*, *3H*, acetate$C\underline{H}_3$), 3.8 (*s*,*3H*, O-$C\underline{H}_3$), 6.36 (*d*, 1H vinyl C_1 H), 6.9-7.0 (*m*, *3H*, methoxyphenyl $C_{2,5,6}$ H), 7.2 (*d*, 2H, *p*-tolyl $C_{3,5}$ H), 7.4 (*d*, 2H, *p*-tolyl $C_{2,6}$ H)7.5 (*t*, quinazolinone C_7 H), 7.76 (*d*, 2H, quinazolinone $C_{6,8}$H), 7.9 (*d*, H, vinyl C_2 H), 8.3 (d, 1H, quinazolinone C_5 H); ^{13}CNMR($CDCl_3$/$CDCl_4$) δppm: 20.70 (1C, *p*-tolyl CH_3), 21.36 (1C, acetate $\underline{C}H_3$), 55.78 (1C, methoxy $\underline{C}H_3$), 111.91 (2C, methoxyphenyl $C_{2,\ 5}$), 119.97 (1 C, methoxyphenyl C_6), 120.69 (1C, quinazolinone C_{4a}), 120.98 (1C, vinyl C_1), 123.15 (2C, *p*-tolyl $C_{2,6}$), 126.64 (1C, quinazolinone C_6), 127.20 (1C, quinazolinone C_8), 127.32 (1C, quinazolinone C_5), 128.39 (1C, *p*-tolyl C_1), 130.60 (2C, *p*-tolyl$C_{3,5}$), 134.27 (1C, *p*-tolyl C_4),134.50 (methoxyphenyl C_1), 134.61 (1 C, quinazolinone C_7), 138.93 (1C, vinyl C_2), 139.38 (1C, methoxyphenyl C_4), 147.76 (1 C, quinazolinone C_{8a}), 151.16 (1C, methoxyphenyl C_3), 151.76 (1C, quinazolinone C_2), 162.36 (1C, quinazolinone C_4) and 168.92 (1C, acetate $\underline{C}$=O). Anal. Calcd. for $C_{26}H_{22}N_2O_4$: C, 73.23; H, 5.20; N, 6.57. Found: C, 72.96; H, 4.91; N, 6.37.

4-[(1*E*)-2-(3,4-dihydro-4-oxo-3-*p*-tolylquinazolin-2-yl)vinyl] phenyl acetate (IVb)

Yield 66.3%; mp 199-201°C; IR (Nujol) (cm^{-1}): 1760 (C=O); 1679 (C=O); 1654 (C=N); 1209 (C-O-C); 1109 (C-O-C); ^{1}HNMR ($CDCl_3$/$CDCl_4$) δppm: 2.3 (*s*, *3H*,*p*-tolyl CH_3), 2.5 (*s*, *3H*, C=OCH_3), 6.35 (*d*, 1H, vinyl C_1 H), 7.04 (*d*, 2H, phenyl acetate $C_{3,5}$ CH), 7.2 (*d*, 2H, *p*-tolyl $C_{3,5}$ H), 7.36 (*d*, 2H, phenyl acetate $C_{2,6}$ H) 7.4 (*d*, 2H, *p*-tolyl$C_{2,6}$H), 7.38 (*t*, quinazolinone C_7 H), 7.77-7.80 (*m*, 2H, quinazolinone $C_{6,8}$ H), 7.94 (*d*, H, vinyl C_2 H), 8.3 (d, 1H, 4(*3H*)-quinazolinone C_5 H); ^{13}CNMR($CDCl_3$/$CDCl_4$) δppm: 18.533 (1C, *p*-tolyl $\underline{C}H_3$), 21.47 (1C, phenyl acetate $\underline{C}H_3$), 120.16 (1C, quinazolinone C_{4a}), 121.04 (2C, *p*-tolyl $C_{2,6}$), 121.97 (2C, phenyl acetate $C_{3,5}$), 126.47 (2C, yphenyl acetate $C_{2,6}$), 127.28 (1C, quinazolinone C_6), 127.38 (1C, quinazolinone C_8), 128.43 (2C, *p*-tolyl $C_{3,5}$), 128.80 (1C, quinazolinone C_5), 130.57 (1C, *p*-tolyl C_1), 133.13 (1 C, *p*-tolyl C_4), 134.39 (1 C, quinazolinone C_7), 134.32 (1C, phenyl acetate C_1), 139.17 (1C, vinyl C_2), 147.72 (1 C, quinazolinone C_{8a}), 151.50 (1 C, quinazolinone C_2), 151.54 (1C, phenyl acetate C_4), 162.14 (1C, quinazolinone C_4) and 168.65 (1C, acetate $\underline{C}$=O). Anal. Calcd. for$C_{25}H_{20}N_2O_3$: C, 77.04; H,5.08; N,7.07. Found: C, 76.82; H, 4.87; N, 7.21.

4-[(1*E*)-2-(3,4-dihydro-4-oxo-3-phenylquinazolin-2-yl) vinyl]-2-methoxy phenyl acetate (IVc)

Yield 48.7%; mp 221-223°C; IR (Nujol) (cm^{-1}): 1759 (C=O); 1676 (C=O); 1637 (C=N); 1201 (C-O-C); 1119 (C-O-C);^{1}HNMR ($CDCl_3$/ $CDCl_4$) δppm:2.3 (*s*,*3H*, –C=$OC\underline{H}_3$), 3.8 (*s*,*3H*, -O-$C\underline{H}_3$), 6.3 (*d*, 1H, vinyl C_1 H), 6.8-7.0 (*s* and 2*d*,*3H*, methoxyphenyl $C_{2,5,6}$ H), 7.35 (*d*, 2H, phenyl $C_{3,5}$ H), 7.5 (t, 1H, quinazolinone C_7 H), 7.56-7.63 (*m*,*3H*, phenyl $C_{2,4,6}$ H), 7.8 (*m*, 2H, quinazolinone $C_{6,8}$ H), 7.9 (*d*, 1H, vinyl C_2 H), 8.3 (*d*, 1H, quinazolinone C_5 H); ^{13}CNMR($CDCl_3$/$CDCl_4$) δppm: 20.61 (1C, -C=OCH_3), 55.58 (1C, -O-CH_3), 111.56 (1C, methoxyphenyl C_2), 120.03 (1C, vinyl C_1), 120.23 (1C, methoxyphenyl C_6), 121.08 (1C, quinazolinone C_{4a}), 123.15 (1C, methoxyphenyl C_5), 126.53 (2C, phenyl $C_{2,6}$), 127.27 (1 C, quinazolinone C_6), 127.39 (1C, phenyl C_4), 128.85 (1C, quinazolinone C_8), 129.21 (2C, phenyl $C_{3,5}$), 129.83 (1C, quinazolinone C_5), 134.29 (1C, methoxyphenyl C_1), 134.43 (1C, phenyl C_1), 137.13 (1C, vinyl C_2), 140.854 (1C, quinazolinone C_{8a}), 147.79 (1C, methoxyphenyl C_4), 151.20 (1C, methoxyphenyl C_3), 151.27 (1C, quinazolinone C_2), 161.93 (1C, quinazolinone C_4) and 168.23 (1C, acetate $\underline{C}$=O).Anal. Calcd. for$C_{25}H_{20}N_2O_4$: C, 72.80; H, 4.89; N, 6.79. Found: C, 73.04; H, 4.71; N, 6.59.

4-[(1*E*)-2-(3,4-dihydro-4-oxo-3-phenylquinazolin-2-yl) vinyl]phenyl acetate (IVd)

Yield 59.5%; mp 221-223°C; IR (Nujol) (cm^{-1}): 1757 (C=O); 1679 (C=O); 1635 (C=N);1209 (C-O-C);1120 (C-O-C);^{1}HNMR ($CDCl_3$/ $CDCl_4$) δppm:2.3 (*s*, *3H*, -C=$OC\underline{H}_3$), 6.3 (*d*, 1H, vinyl C_1 H), 7.05 (*d*, 2H, phenyl acetate $C_{3,5}$ H), 7.30-7.35 (*m*, 4H, phenyl $C_{3,5}$H and phenyl acetate $C_{2,6}$ H), 7.50 (*t*, 1H, quinazolinone C_7 H), 7.56-7.63 (*m*, *3H*, phenyl $C_{2,4,6}$H), 7.75-7.84 (*m*, 2H,quinazolinone $C_{6,8}$ H), 7.9 (*d*, 1H, vinyl C_2 H), 8.3 (*d*, 1H, quinazolinone C_5 H);^{13}CNMR ($CDCl_3$/$CDCl_4$) δppm: 21.08 (1C, acetate $C\underline{H}_3$), 120.00(1C, vinyl C_2), 120.05 (2C, phenyl $C_{2,6}$), 121.98 (1C, quinazolinone C_{4a}), 126.51 (2C, phenyl acetate $C_{3,5}$), 127.25 (1 C, quinazolinone C_6), 127.42 (1C, phenyl C_4), 128.75 (1C, quinazolinone C_8), 128.79 (2C, phenyl acetate $C_{2,6}$), 129.30 (2C, phenyl $C_{3,5}$), 129.87 (1C, quinazolinone C_5),133.02 (1C, phenyl C_1), 134.42 (1C, quinazolinone C_7), 137.07 (1C, phenyl acetate C_1), 138.74 (1C, vinyl C_2), 147.79 (1C, quinazolinone C_{4a}), 151.31 (1C, phenyl acetate C_4), 151.55 (1C, quinazolinone C_2), 161.97 (1C, quinazolinone C_4,$\underline{C}$=O)and 168.56 (1C, acetate $\underline{C}$=O). Anal. Calcd. for$C_{24}H_{18}N_2O_3$: C,75.38; H,4.74; N,7.33. Found: C, 75.52; H, 4.46, N7.60.

2-(2-Nitrostyryl)-3-phenyl-4(*3H*)-quinazolinone (IVe)

Yield 74%; mp 197-199°C; IR (Nujol) cm^{-1}: 1677 (C=O); 1630 (C=N); 1603 and 1377 (NO_2);^{1}HNMR ($CDCl_3$/$CDCl_4$) δppm: 6.3 (*d*, 1H, vinyl C_1 H), 7.28 (*d*, 1H, phenyl C_4 H), 7.35 (*d*, 2H, phenyl $C_{2,6}$H), 7.46 (*t*, 1H, quinazolinone C_7 H), 7.48-7.57 (*m*, *3H*,*o*-nitrophenyl $C_{4,5,6}$ H), 7.60 (*t*, 2H, phenyl $C_{3,5}$ H), 7.8 (*d*, 2H, quinazolinone $C_{6,8}$ H),

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7.9 (*d*, 1H, vinyl C_2 H), 8.3 (*d*, 1H, quinazolinone C_5 H), 8.4(*d*, 1H, *o*-nitrophenyl C_3 H); ^{13}CNMR ($CDCl_3$/$CDCl_4$) δppm: 121.19 (1C, vinyl C_2), 124.63 (1C, quinazolinone C_{4a}), 124.84 (C, *o*-nitrophenyl C_3), 126.99 (2C, phenyl $C_{2,6}$), 127.15 (1 C, quinazolinone C_6), 128.74 (1C, phenyl C_4), 128.80 (1C, quinazolinone C_8), 129.35 (2C, *o*-nitrophenyl C_6), 129.46 (2C, phenyl $C_{3,5}$), 129.92 (1C, quinazolinone C_5), 131.45 (1C, *o*-nitrophenyl C_4), 133.06 (1C, quinazolinone C_7), 134.51 (1C, *o*-nitrophenyl C_1), 134.92 (1C, phenyl C_1), 136.89 (1C, vinyl C_2), 147.56 (1C, quinazolinone C_{8a}), 148.47 (1C, *o*-nitrophenyl C_2), 150.32 (1C, quinazolinone C_2)and 161.82 (1C, quinazolinone $C_{4,}$C=O). Anal. Calcd. for $C_{22}H_{15}N_3O_3$:C, l 71.54; H,4.09; N,11.38. Found: C, 71.82; H, 3.86; N, 11.67.

2-(2-nitrostyryl)-3-anilinoquinazolin-4(*3H*)-one (IVf)

Yield 92.1%; mp 201-203°C; IR (Nujol) (cm^{-1}): 3254 (NH); 1682 (C=O); 1630 (C=N); 1603 and 1377 (NO_2); ^{1}HNMR ($CDCl_3$/$CDCl_4$/ MeOD-d_4) δppm: 6.85 (*d*, 1H, vinyl C_1 H), 7.02 (*t*, 1H, anilino C_4 H), 7.27 (*t*,*3H*, anilino $C_{3,5}$H and *o*-nitrophenylC_4H), 7.35 (*s*, 1H, anilino NH), 7.48-7.57 (*m*,*3H*, *o*-nitrophenyl $C_{4,5,6}$ H), 7.6 (*t*, 1H, quinazolinone C_7 H), 7.68 (*d*, 1H, *o*-nitrophenyl C_5 H), 7.80-7.84 (*m*, 2H, quinazolinone $C_{6,8}$ H), 8.05 (*d*, 1H, vinyl C_2 H), 8.3 (*d*, 1H, quinazolinone C_5 H), 8.6 (*d*, 1H, *o*-nitrophenyl C_3 H).Anal. Calcd. for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.57; H, 5.52; N, 16.91.

Anti-Malarial Screening

Experimental animals

Swiss albino mice of both sexes, weighing 25-35 g and aged 6-8 weeks purchased from Ethiopian Health and Nutrition Institute were used in the study. The mice were acclimatized to the laboratory conditions (temperature of 23-25°C with average relative humidity of 60%) for a period of 7 days before use. The mice were housed in standard cages and maintained on standard pelleted diet and water.

The *Plasmodium berghei* parasite

The rodent malaria parasite, *P. berghei* ANKA ((chloroquine sensitive) strain, obtained from the Biomedical lab at the Department of Biology, Faculty of Science, Addis Ababa University, Ethiopia was used to infect the mice for a four-day suppressive test.

In vivo antimalarial activity

In vivo antimalarial activity test of the synthesized compounds was performed using a 4-day standard suppressive test [23].On day 0, the test mice were injected with 0.2 ml of 2×10^7 parasitized erythrocytes, (*P. berghei* ANKA strain) intravenously. After 2 hr, the infected mice were weighed and randomly divided into five groups of five mice per cage. Groups 1, 2 and 3 received the synthesized compounds at 20 mg/kg and 40 mg/kg dose levels and served as treatment groups [24]. Group 3 received the vehicle (7% Tween 80, 3% ethanol in water) and served as negative control. Group 4 received the standard drug chloroquine phosphate (20 mg/Kg) and served as positive control [25].

On days 1 to 3, animals in the experimental groups were treated again (with the same dose of the synthesized compound and same route daily) as in day 0. On day 4 (i.e. 24 hr after the last dose or 96 hr post-infection), blood smear from all test animals was prepared using Giemsa stain. Level of parasetimia was determined microscopically by counting 4 fields of approximately 100 erythrocytes per field. The difference between the mean value for the negative control group (taken as 100%) and those of the experimental groups was calculated and expressed as percent suppression or activity.

Untreated control mice typically die in about one week after infection [26]. For treated mice the survival-time (in days) was recorded and the mean survival time was calculated in comparison with that of the negative group [27].

In vivo acute toxicity

Oral acute toxicity study was done for the synthesized compounds. Four groups of mice, each group consisting of six male mice were used for testing acute toxicity. The mice in each group were fasted over night and weighed before test. Test compounds were dissolved in 70% Tween 80 and 30% ethanol. This solution was further diluted 10-fold with sterile distilled water to give a stock solution containing 7% Tween 80 and 3% ethanol [28]. Mice in groups one, two and three were given 100, 250 and 500 mg/kg/day of the synthesized compounds respectively with a maximum dose volume of 1 ml/100 g of body weight orally while mice in the control group (group four) were treated with the vehicle. After administration of the substance food was withheld for a further 2 hr period [29]. Toxicity signs such as changes in skin, eyes (blinking), tremors, convulsion, lacrimation, muscle weakness, sedation, urination, salivation, diarrhea, lethargy, sleep, coma and also death were observed for 72 hrs. Twenty-four hours later, the % mortality and weight of mice in each group and for each test compound at each dose level were recorded [30].

Data analysis

Results of the study were expressed as mean ± standard deviation. Statistical significance for suppressive test was determined by one-way ANOVA at 95% confidence limits (p=0.05). Data on body weight and survival time were analyzed. All the data were analyzed using Microsoft office excel 2007.

Percentage parasitaemia and percentage suppression were calculated using the following formulae:

$$\%\text{Paracetaemia} = \frac{\text{Number of infected RBC}}{\text{Number of total RBC}} \times 100$$

$$\%\text{Paracetaemia} = \frac{\text{Paracetaemia in negative control-Parastemia in trestement group RBC}}{\text{Paracetaemia in negative control}} \times 100$$

Chemistry

The required starting 2-methyl-3,1-benzoxazin-4-one **II**, was synthesized from anthranilic acid **I** and acetic anhydride [31]. 2-Methyl-3-aryl-*3H*-quinazolin-4-ones **IIa-c,**was prepared according to reported methods [24]. A mixture of acetanthranil **II**, (15.67g, 0.1 mole) and an equimolar amount of the appropriate aromatic amine was heated under reflux for 5-7 hrs. The 3-aryl-2-(substituted styryl)-4(*3H*)-quinazolinones **IVa-f**, were prepared by Perkins condensation of **IIIa-c** with substituted aromatic aldehydes [32]. A mixture of 3-aryl-2-methyl-4(*3H*)-quinazolinone **IIIa-c,** (10 m mole) and an equimolar amount of the appropriate aromatic aldehyde was allowed to react in the presence of fused sodium acetate by heating under reflux for 10-12 hrs.

Results and Discussion

A series of novel 2,3-disubstituted styryl-4(*3H*)-quinazolinone derivatives **IVa-f** were prepared in good yields. The synthetic routes to these compounds are given in Figure 1. The structures of the compounds were verified based on data from elemental analysis, IR, ^{1}HNMR and ^{13}CNMR spectral studies.

IR studies

The summarized characteristic stretching and bending IR vibration

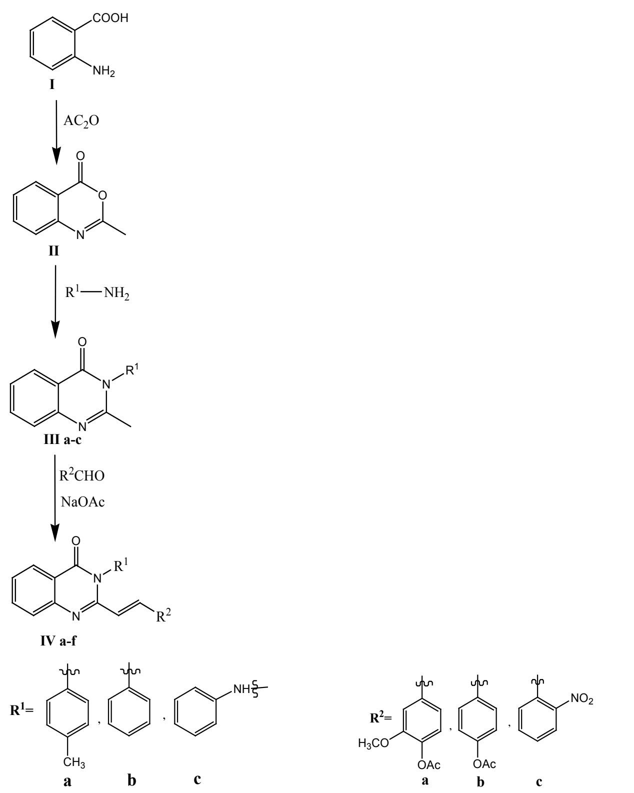

Figure 1: Synthetic routes to the compounds

frequencies of important functional groups are discussed below. The IR spectrum of compound **IIIc** showed a characteristic absorption band at 3264 cm^{-1}(-NH), 1675 cm^{-1}(>C=O) and at 1649 cm^{-1}(>C=N).The appearance of a band at 1760 cm^{-1} (>C=O) is evident for the presence of acetate in compound **IVa** and other characteristic absorption bands appeared at 1670 (>C=N),1655 cm^{-1}(>C=O).The other bands appeared at 1220 and 1119 cm^{-1} were attributed to the ester group. Characteristic IR absorption bands of compound **IVb** appeared at 1760 cm^{-1}(>C=O), 679 cm^{-1}(>C=O), 1654 cm^{-1}(>C=N), 1209 and 1109 cm^{-1}(C-O-C).Compound **IVc** showed a sharp absorption band that at 1759 cm^{-1}(>C=O), 1676 cm^{-1}(>C=O), 1637 cm^{-1}(>C=N), 1201 and 1119 cm^{-1}(C-O-C).Where as IR absorption bands of compound **IVd** appeared at 1757 cm^{-1}(>C=O), 1679 cm^{-1}(>C=O) 1635 cm^{-1}(>C=N), 1209 and 1120 cm^{-1}(C-O-C).The characteristic absorption bands of**IVe** appeared at 1677 cm^{-1}(>C=O), 1630 cm^{-1}(>C=N), 1603 and 1377 cm^{-1}(-NO_2).On the other hand the IR spectrum of compound **IVf** showed a sharp absorption band at 3254 cm^{-1}(-N-H),1682 cm^{-1}(>C=O), 1630 cm^{-1}(>C=N).

NMR studies

The ^{1}HNMR spectrum of the compound **IIIc** showed a doublet at δ 8.15 ppm which is representative for the C_5proton of 4(*3H*)-quinazolinone. A doublet at δ 6.56 ppm, and a multiplate at δ 6.8 ppm and δ7.1 ppm indicated the presence of phenyl ring. The appearance of three singlets at δ 2.3, δ 2.5 and δ 3.8 ppm in the^{1}HNMR spectra of **Iva** clearly indicates the *p*-tolyl, acetate and methoxy moieties respectively. The doublets at δ 6.4 ppm and δ 7.9 ppm confirms for the presence of styryl vinilic protons. The peaks characteristic to the 4(*3H*)-quinazolinone moiety appeared at δ 8.3 ppm, δ 7.5 ppm, and 7.8 ppm.The disappearance of signals corresponding to the protons of the methoxy group and appearance of multiplets for aromatic proton in the range of δ 6.4 ppm-8.3 ppm clearly indicate the formation of **IVb**. In the^{1}HNMR spectrumof compound **IVc** peaks that appeared at δ 7.35 ppm and δ 7.56-7.63 ppm denotethe five phenyl protons.The absence of proton signals of the methoxy groupin the ^{1}HNMR spectrum of **IVd** and the appearance of all the other peaks provedthe formation of **IVd.**The appearance of an unusual peak at δ 8.4 ppm in the ^{1}HNMR spectrum of compound **IVe** is due to the proton *ortho* to the NO_2. The presence of a singlet peak at 7.4 ppm in the ^{1}HNMR spectrum of compound **IVf** is due to the NH group and other peaks at δ 7.02 ppm, δ 7.27 ppm,δ 7.48-7.57 ppm, δ 7.68 ppmand δ 8.6 ppm were attributed to the nine aromatic protons of the anilino and the *o*-nitrostyryl groups.

Another strong support for the structures of IIIc, IVa, IVb, IVc, IVd, and IVe is the 13C NMR spectra. The 13C NMR spectra of compounds were taken in $CDCl_3$/CCl_4 and the signals obtained were all in a good agreement with the proposed structures. The carbonyl and imine carbons of quinazolinone ring are resonates at about d 170.0 ppm and 160.1 ppm respectively for all of the compounds. In addition to this the ^{1}H-^{1}H COSY, DEPT-135, HMBC and HSQC spectra of compound IVa were observed.

Biological Screening

In vivo antimalarial activity

To ascertain their antimalarial activity, compounds **IIIc, IVa, IVb, IVc, IVd, IVe** and **IVf** were assayed *in vivo* against *P. berghei*, a rodent malaria parasite, using a 4-day standard suppressive test [26]. The synthesized compounds were given at dose levels of 20 mg/kg and then 40 mg/kg to see if there is a dose-response relationship (Table 1). A dose of 20 mg/kg was considered to be the initial low dose based on previous works done for other synthesized compounds [27].

One-way ANOVA of the percent suppression of the negative control with groups that received the test compounds revealed that only compounds **IVc** and **IVe** showed statistically significant percent suppression at 20 mg/kg at 95% confidence limits (P=0.05). On the other hand among the groups that received the test compounds at a dose level of 40 mg/kg the result revealed that compounds **IIIc**, **IVa**, **IVb** and **IVf** exhibited statistically significant percent suppression. In general, the compounds **IIIc**, **IVa, IVb,** and **IVf** having secondary amine group,2-nitrostyryl group and methyl group of *p*-tolyl substituent showed very promising activity with percent suppression of 70.7, 78.4, 60.6 and 71.6 at 40 mg/kg *in vivo* against *P. berghei* as compared to the standard drug chloroquine phosphate. Whereas the rest of the compounds showed moderate and lower activity against *P. berghei* as compared to the standard drug chloroquine phosphate as shown in Table 1. None of the compounds were as equipotent as the standard drug chloroquine phosphate. The data also revealed that the activity of compound **IVa>IVf>IIIc>IVb**. Therefore, it can be inferred that presence of polar and non-planar substituents imparts much towards antimalarial power of these compounds.

In vivo acute toxicity

A preliminary toxicity test was conducted to assess the acute lethal, physical and behavioral effects of the synthesized compounds after oral administration to mice. The toxicity study was designed to verify whether the synthesized compounds are safe to be used for subsequent experiments or not. Acute toxicity study was performed for the two most active compounds **IVa** and **IVf** that showed good activity when given at both dose levels. Oral administration of the two relatively active compounds in doses of 100, 250 and 500 mg/kg did not produce any significant acute toxic effects on the experimental mice.

Gross behavioral and physical observations like hair erection, urination, muscle weakness, sedation and convulsion, reduction in feeding activity in the test mice were used as indicators of acute toxicity effects. The test mice monitored once daily for 14 days did not show any of the signs of toxicity mentioned. None of the test animals died within the first twenty-four hour. The data indicated that the test compounds did not show any significant change in weight and the slight variation observed was not found to be dose dependent (Table 2).

The results of the study showed no adverse effects for the synthesized compounds indicating that the median lethal dose (LD_{50}) of synthesized compounds could be much higher than 500 mg/kg/day for mice through oral route. The results indicated that test compounds proved to be non-toxic and well tolerated by the experimental animals up to 500 mg/kg.

Conclusion

In this study, a number of structurally related 4(*3H*)-quinazolinone derivatives were synthesized. Structures of the synthesized compounds were confirmed by various spectroscopic methods and elemental microanalyses. All the target compounds were efficiently synthesized in good yield and purity.

One representative intermediate and six target compounds were screened for their antimlarial activity in *P. berghei* infected mice. The result revealed that four of the synthesized compounds **IIIc**, **IVa**, **IVb** and **IVf** were having a promising antimalarial activity (60.6-78.4% suppression of the parasitaemia), but none of the compounds were as active as the reference standard drug, chloroquine, in the doses tested. Compounds **IVc** and **IVe** showed significant suppression at initial low dose (20 mg/kg) while compound **IVd** showed a very week activity at

Test substance	Dose mg/kg	% Parasitaemia	% Suppression	Mean survival time (Days)
IIIc	20	58.2 ± 0.2	15.5	8.5 ± 0.6
	40	27.4 ± 1.1	70.7	9.0 ± 0.5
IVa	20	49.8 ± 0.2	27.7	9.2 ± 0.6
	40	20.2 ± 2.0	78.4	9.8 ± 0.4
IVb	20	61.6 ± 0.1	10.6	8.5 ± 1.0
	40	36.9 ± 2.0	60.6	9.3 ± 0.6
IVc	20	41.8 ± 0.2	39.3	9.0 ± 0.8
	40	86.8 ± 0.6	7.3	7.7 ± 0.6
IVd	20	48.8 ± 0.1	29.2	7.6 ± 0.5
	40	89.2 ± 2.5	4.7	6.7 ± 1.2
IVe	20	44.5 ± 0.1	35.4	9.7 ± 0.5
	40	90.6 ± 1.7	3.2	7.3 ± 0.5
IVf	20	51.8 ± 2.2	24.8	8.3 ± 0.6
	40	26.6 ± 0.5	71.6	8.5 ± 0.6
NC (20)	1 ml/100g	68.9 ± 0.1	0.0	6.4 ± 0.5
NC (40)	1 ml/100 g	93.6 ± 0.1	0.0	6.6 ± 0.5
Chloroquine	20 mg/kg	0.0	100	ND

*Values are M ± SD, P<0.05, NC: Negative control, ND: No death recorded over the experimental period.

Table 1: Antiplasmodial activities of the synthesized compounds at 20 mg/kg and at 40 mg/kg*

Test substances	Dose mg/Kg	Wt. before test	Wt. after test
IVa	100	26.6 ± 1.3	25.7 ± 1.5
	250	23.0 ± 1.3	25.7 ± 1.1
	500	25.0 ± 1.4	25.4 ± 1.8
IVf	100	27.1 ± 1.4	29.4 ± 1.7
	250	26.1 ± 1.7	26.5 ± 1.4
	500	28.3 ± 1.6	29.5 ± 1.8
NC	1 ml/100g	28.2 ± 1.7	27.3 ± 1.5

* Key: Values are M ± SD

Table 2: Result of acute toxicity studies*

both dose levels tested.

Oral acute toxicity profile was determined for the two most active compounds, **IIIc** and **IVa**. The result revealed that oral administration of the synthesized compounds in single doses (100, 250 and 500 mg/kg) had no adverse effects, indicating that the compounds may have high safety margin and their LD_{50} could be higher than 500 mg/kg. Since the 4(*3H*)-quinazolinone derivatives have not been used as antimalarial agents the existence of resistance against these compounds is barely true. Therefore these 4(*3H*)-quinazolinone derivatives are expected to have activity against plasmodium species resistant to existing drugs.

Recommendation

In this work only limited numbers of compounds were synthesized and their antimalarial activity was tested. Thus more compounds should be synthesised and tested to exploit the possible most active 4(*3H*)-quinazolinone derivatives. Additional toxicity studies need to be done to prove the sub-acute and chronic safety of the synthesized compounds. The antimalarial mechanism of action of 4(*3H*)-quinazolinone derivativesneed to be determined and docking study be performed.

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Conflict of Interests

The authors declare no conflict of interests.

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