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Natural Product Inspired Synthesis of Tryptanthrin Analogues as Potential Antimalarial Agents

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ABSTRACT

A new series of tryptanthrin analogues have been synthesized as potential antimalarial molecules. Synthesis of tryptanthrin aminoalkyl derivatives have been achieved *via* alkylation of oxime functionality of tryptanthrin derivatives by various alkyl amino pharmacophoric chains. A series of 21 tryptanthrin aminoalkyl analogues were synthesized with variation in both parent natural alkaloid and in aminoalkyl side chains. Synthesized compounds were fully characterized with ^{1}H & ^{13}C NMR, IR spectroscopy. Further all the members were screened for their antimalarial potential against *Plasmoum falciparum* in both sensitive (3D7) and in resistant (k1) strains. Most of the screened compounds were exhibited potent antimalarial activity in both strains. Compounds (**5m**, **3c** and **5l**) having nitro group at the 8 position in tryptanthrin framework were most promising compounds in series (IC_{50} = 10 nm) with IC_{50} value as low as 10 nm comparable to chloroquine. These compounds were also tested for their toxic effect and found to be highly safe with high value of SI index.

KEYWORDS

Antimalarial, Tryptanthrin, Natural product, Animo alkyl chains, Oximes.

INTRODUCTION

Malaria represents one of the most common public health issues which is responsible for deaths of about one billion people during the last two centuries, mostly among African children [1-4]. *Plasmodium falciparum* is the largely fatal among four species of Plasmodium causing malaria in humans and account for most of the deaths from infectious diseases [5]. The severity of disease is further extended due to the rising cases of resistance towards existing drugs. Due this situation medicinal chemists are under substantial pressure to develop new chemotherapeutics against this disease quickly [6,7]. In spite of all efforts in drug development against malaria, the disease has been spreading at a steady rate over the past decade. To combat the development of resistance towards the mainstream drugs such as atovaquone, sulfadoxine, pyrimethamine, mefloquine and also artemisinin, in pathogen is also a major hurdle for scientists and researchers [8-10]. So, there is an urgent need for the development of new therapeutics which are effective to tackle drug resistance and have higher efficacy for the treatment of malaria especially in the developing countries.

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Historically, natural products and natural products inspired molecules have made immense contribution to malaria chemotherapy either directly as antimalarial agents or as important lead compounds for the discovery of more potent antimalarials [11-14]. The importance of natural molecules as chemotherapeutic agents lies in their unique chemical biodiversity, inherent stability, drug-like properties and, often their structural adaptation to target proteins in biological system [15,16]. One of the most primitive natural compounds that draw attention to the value of natural products in the battle against malaria is quinine [17], isolated from the Cinchona bark. It also served as a template for the development of structurally simpler analogues such as chloroquine, primaquine, mepacrine and mefloquine that served as effective antimalarials [18-20]. The more recent example among the antimalarial natural products, whose diverse pharmacological potential has captivated the scientific community, is artemisinin isolated from the Chinese plant *Artemesia annua* [20-22]. These antimalarial natural products briefly highlighted above, clearly demonstrate the huge potential that natural products hold in providing powerful lead structures for the development of antimalarial agents [23].

Tryptanthrin (indolo[2,1-*b*]quinazoline-6,12-dione) is a basic alkaloid found in a number of plant species [24]. It is active principal component of a traditional Japanese herbal remedy for fungal infections [25]. Tryptanthrin is a compound with a long history and is well documented to possess antibacterial activity against variety of pathogenic bacteria, particularly the causative agent of tuberculosis [26]. Tryptanthrin and derivatives are also well known as potential anticancer agents against MCF-7, NCI-H460 and SF-268 human cancer cell lines [27-29]. Tryptanthrin have quinazolines and indole moieties in their core structure. The quinazoline core is a building block for approximately 150 naturally occurring alkaloids isolated from a number of families of plant kingdom [30]. In continuation of this, we have earlier reported the first green synthesis of tryptanthrins [31]. These pharmacological properties associated with tryptanthrin and importance of quinazoline nucleus in medicinal chemistry prompted us to synthesize tryptanthrin derivatives as therapeutic agents. This time, we hypothesized to synthesize tryptanthrin nucleus and ligate them with different pharmacophoric aminoalky groups in order to evaluate their antimalarial potential.

EXPERIMENTAL

All the reactions were carried out at room temperature around 28-30 ºC. Unless otherwise specified, all the reagents were purchased from Sigma-Aldrich Chemical Co, Lancaster and used directly without further any purification. NMR spectra were obtained using the Brucker DRX 300 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to TMS, coupling constants (*J*) in Hz. IR spectra were taken on VARIAN FT-IR spectrometer as KBr pellets (when solid). Elemental analysis was performed using a Perkin-Elmer Autosystem XL Analyzer. Melting points were measured using a COMPLAB melting-point apparatus. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates visualized with UV light.

General method for synthesis of tryptanthrin oxime derivatives (4a-c): Tryptanthrin analogues (1 eq.), hydroxyamine hydrochloride (1.5 eq.) and sodium hydroxide (1.5 eq.) were mixed in 100 mL round bottom flask in 20 mL of toluene and refluxed upto completion of reaction. Progress of reaction was checked by TLC. After completion of reaction solvent was evaporated under reduced pressure and crude mass was extracted with ethyl acetate and water. Organic layer was separated and dried over sodium sulfate and purified by coloumn chromatography using dichloromethane as mobile phase. The resulting oxime derivative was isolated as green solid.

General method for synthesis of tryptanthrin aminoalkyl derivatives (5a-o): Tryptanthrin oxime derivative (1 eq.), sodium hydride 60% in paraffin (2 eq.) were taken in 15 mL DMF in 100 mL round bottom flask. Alkylamino chain (1.2 eq.) was added and stirred at room temperature upto completion of reaction. After completion crude reaction mass was extracted using ethyl acetate and water. Organic Phase separated, dried over sodium sulphate and solvent was evaporated under reduced pressure. Oily residue isolated by coloumn chromatography using chloroform and methanol mixture as mobile phase. Finally, the desired product was isolated as viscous oil.

Indolo[2,1-*b*]quinazoline-6,12-dione (3a): Green solid; m.p.: 258 ºC; ^{1}H NMR (300 MHz $CDCl_3$) = 8.61 (d, 1H, *J* = 3.4 Hz), 8.44 (d, 1H, *J* = 1.17 Hz), 8.06 (d, 1H, *J* = 8.01 Hz), 7.94-7.80 (m, 3H), 7.69 (t, 1H, *J* = 7.08 Hz), 7.28 (t, 1H, *J* = 7.44 Hz); ^{13}C NMR (75 MHz $CDCl_3$) δ = 117.5, 120.6, 125.4, 126.3, 127.1, 129.7, 130.0, 133.2, 134.6, 145.3, 146.6, 160.4, 183.8; ESMS (*m*/*z*): 249 $(M+H)^+$; Anal. calcd. (found) % for $C_{15}H_8N_2O_2$: C, 72.58 (72.55); H, 3.25 (3.21); N, 11.28 (11.31).

8-Chloroindolo[2,1-*b*]quinazoline-6,12-dione (3b): Green solid; m.p.: 234 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.62 (d, 1H, *J* = 6.8 Hz), 8.44 (d, 1H, *J* = 5.8 Hz), 8.06 (d, 1H, *J* = 7.15 Hz), 7.90-7.88 (m, 2H), 7.76-7.68 (m, 2H); ^{13}C NMR (75 MHz $CDCl_3$) δ = 116.5, 121.8, 123.5, 127.6, 127.9, 128.3, 130.0, 133.9, 134.4, 141.3, 146.5, 148.6, 152.7, 161.6, 188.4; ESMS (*m*/*z*): 283 $(M+H)^+$; Anal. calcd. (found) % for $C_{15}H_7N_2O_2Cl$: C, 63.73 (63.70); H, 2.50 (2.46); N, 9.91 (9.94).

8-Nitroindolo[2,1-*b*]quinazoline-6,12-dione (3c): Green solid; m.p.: > 260 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.74-8.72 (m, 2H), 8.54 (s, 1H), 8.39 (d, 1H, *J* = 6.0), 7.79 (d, 2H, *J* = 3.14 Hz), 7.77-7.74 (m, 1H); ^{13}C NMR (75 MHz $CDCl_3$) δ = 115.0, 120.0, 120.8, 123.8, 126.6, 127.3, 133.4, 145.5, 147.1, 153.9, 160.6, 186.4; ESMS (*m*/*z*): 294 $(M+H)^+$; Anal. calcd. (found) % for $C_{15}H_7N_3O_4$: C, 61.44 (61.41); H, 2.41 (2.37); N, 14.33 (14.36).

(*E*)-6-(Hydroxyimino)indolo[2,1-*b*]quinazolin-12(6*H*)-one (4a): Green solid; m.p.: 251 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.59 (d, 1H, *J* = 4.4 Hz), 8.42-8.29 (m, 2H), 7.89-7.80 (m, 2H), 7.68-7.48 (m, 2H), 7.43 (t, 1H, *J* = 7.00Hz), 2.50 (s, 1H); ^{13}C NMR (75 MHz $CDCl_3$) δ = 117.2, 120.6, 124.3, 126.3, 127.1, 129.7, 130.0, 133.2, 134.6, 145.3, 146.6, 158.6, 183.8; ESMS (*m*/*z*): 384 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{15}N_2O_2Cl$: C, 67.36 (67.31); H, 4.46 (4.42); N, 8.27 (8.29).

(*E*)-8-Chloro-6-(hydroxyimino)indolo[2,1-*b*]quinazolin-12(6*H*)-one (4b): Green solid; m.p.: 243 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.60 (d, 1H, *J* = 7.2Hz), 8.46 (d, 1H, *J* = 6.4 Hz), 8.10 (d, 1H, *J* = 7.1 Hz), 7.94-7.85 (m, 2H), 7.74-7.69

(m, 2H), 2.54 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 117.0, 121.6, 124.1, 124.4, 127.2, 127.3, 128.1, 130.3, 133.4, 133.2, 140.6, 144.3, 146.2, 152.4, 160.2, 188.2; ESMS (*m*/*z*): 298 $(M+H)^+$; Anal. calcd. (found) % for $C_{15}H_8N_3O_2Cl$: C, 60.52 (60.48); H, 2.71 (2.68); N, 14.12 (14.16).

(*E*)-6-(Hydroxyimino)-8-nitroindolo[2,1-*b*]quinazolin-12(6*H*)-one (4c): Green solid; m.p.: 260 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.72-8.70 (m, 2H), 8.51 (s, 1H), 8.34 (d, 1H, *J* = 7.2), 7.79 (d, 2H, *J* = 3.1 Hz), 7.76-7.71 (m, 1H), 2.58 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 116.2, 120.1, 120.8, 123.6, 126.6, 132.2, 133.4, 145.5, 147.4, 153.9, 158.8, 186.4; ESMS (*m*/*z*): 309 $(M+H)^+$; Anal. calcd. (found) % for $C_{15}H_8N_4O_4$: C, 58.45 (58.42); H, 2.62 (2.58); N, 18.18 (18.21).

(*E*)-6-(2-(Piperidin-1-yl)ethoxyimino)indolo[2,1-*b*]-quinazolin-12(6*H*)-one (5a): Pale yellow viscous oil, ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.68 (1 H, d, *J* = 8.0 Hz), 8.44 (1H, d, *J* = 7.9 Hz), 8.35 (1H, d, *J* = 7.5 Hz), 7.98 (1H, d, *J* = 8.0 Hz), 7.88-7.75 (1 H, m), 7.64-7.52 (2H, m), 7.38 (1 H, t, *J* = 7.58 Hz), 4.76 (2H, t, *J* = 7.6 Hz), 2.89 (2H, t, *J* = 5.8 Hz), 2.53 (4H, t, *J* = 5.4 Hz), 1.75-1.56 (m, 4H), 1.46-1.44 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 28.6, 41.3, 64.8, 117.4, 121.1, 124.6, 127.2, 127.1, 129.5, 130.8, 133.8, 135.7, 144.1, 146.2, 157.9, 183.6; ES-MS (*m*/*z*): 375 $(M+H)^+$; Anal. calcd. (found) % for $C_{22}H_{22}N_4O_2$: C, 70.57 (70.53); H, 5.92 (5.88); N, 14.96 (14.97).

(*E*)-6-(2-(pyrrolidin-1-yl)ethoxyimino)indolo[2,1-*b*]-quinazolin-12(6*H*)-one (5b): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.67 (1 H, d, *J* = 8.4 Hz), 8.46 (1H, d, *J* = 7.9 Hz), 8.45 (1H, d, *J* = 7.5 Hz), 7.99 (1H, d, *J* = 8.07 Hz), 7.84-7.79 (1H, m), 7.65-7.56 (2H, m), 7.41 (1H, t, *J* = 7.6 Hz), 4.84 (2H, t, *J* = 5.7 Hz), 3.13 (2H, t, *J* = 5.7 Hz), 2.70-2.76 (4H, m), 1.88-1.70 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 23.4, 54.6, 56.2, 67.8, 117.4, 121.0, 124.6, 126.2, 127.4, 129.4, 131.1, 133.4, 134.6, 146.1, 146.6, 157.6, 183.8; ES-MS (*m*/*z*): 361 $(M+H)^+$; Anal. calcd. (found) % for $C_{21}H_{20}N_4O_2$: C, 69.98 (69.94); H, 5.59 (5.54); N, 15.55 (15.58).

(*E*)-6-((3-Chloropropoxy)methylene)indolo[2,1-*b*]-quinazolin-12(6*H*)-one (5c): Faint white solid; m.p.: 188 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.68 (1 H, d, *J* = 8.3 Hz), 8.48 (1H, d, *J* = 7.4 Hz), 8.27 (1H, d, *J* = 7.6 Hz) 7.99 (1H, d, *J* = 8.0 Hz), 7.84-7.78 (1 H, m), 7.76-7.56 (2H, m), 7.40 (t, 1H, *J* = 7.7 Hz), 4.80 (2H, t, *J* = 6.0 Hz), 3.75 (2H, t, *J* = 6.4 Hz), 2.42-2.38 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 29.2, 41.4, 65.1, 117.1, 121.0, 124.6, 127.4, 127.1, 140.5, 130.8, 133.8, 135.7, 144.1, 146.2, 157.9, 183.6; ESMS (*m*/*z*): 339 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{15}N_2O_2Cl$: C, 67.36 (67.31); H, 4.46 (4.42); N, 8.27 (8.29).

(*E*)-6-(2-(Diisopropylamino)ethoxyimino)indolo[2,1-*b*]quinazolin-12(6*H*)-one (5d): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.61 (1 H, d, *J* = 8.0 Hz), 8.48 (1H, d, *J* = 7.9 Hz), 8.37 (1H, d, *J* = 7.5Hz), 7.98 (1H, d, *J* = 7.86 Hz), 7.86-7.75 (1H, t, *J* = 6.8 Hz), 7.60 (2H, q, *J* = 7.4 Hz), 7.41 (1H, t, *J* = 7.5 Hz), 4.60 (2H, t, *J* = 6.7 Hz), 3.12-3.67 (2H, m), 2.96 (2H, t, *J* = 6.6 Hz), 1.07 (s, 1H), 1.05 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.8, 41.6, 52.1, 64.8, 117.4, 121.1, 124.6, 127.2, 127.1, 129.5, 130.8, 133.8, 135.7, 144.1, 146.2, 157.9, 183.6; ES-MS (*m*/*z*): 391 $(M+H)^+$; Anal. calcd. (found) % for $C_{23}H_{26}N_4O_2$: C, 70.75 (70.71); H, 6.71 (6.68); N, 14.35 (14.38).

(*E*)-6-(2-(Dimethylamino)ethoxyimino)indolo[2,1-*b*]-quinazolin-12(6*H*)-one (5e): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.71 (1 H, d, *J* = 8.3 Hz), 8.62 (1H, d, *J* = 8.0 Hz), 8.33 (1H, d, *J* = 7.6 Hz), 8.00 (1H, d, *J* = 7.1 Hz), 7.84-7.61 (1H, m), 7.61-7.56 (2H, m), 7.40 (1H, t, *J* = 6.9 Hz), 4.76 (2H, t, *J* = 5.8 Hz), 2.89 (2H, t, *J* = 5.7 Hz), 2.41 (6*H*, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 44.2, 56.2, 68.8, 117.3, 121.0, 124.6, 126.9, 127.1, 129.5, 130.6, 133.8, 134.7, 144.1, 146.2, 157.1, 183.4; ES-MS (*m*/*z*): 335 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{18}N_4O_2$: C, 68.25 (68.21); H, 5.43 (5.40); N, 16.76 (16.79).

(*E*)-8-Chloro-6-(2-(piperidin-1-yl)ethoxyimino)indolo-[2,1-*b*]quinazolin-12(6*H*)-one (5f): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.62 (1 H, d, *J* = 7.4 Hz), 8.46 (1H, d, *J* = 7.8 Hz), 8.33 (1H, s), 7.96 (1H, d, *J* = 8.0 Hz), 7.62-7.56 (2H, m), 7.37 (1 H, t, *J* = 7.58 Hz), 4.75 (2H, t, *J* = 7.6 Hz), 2.90 (2H, t, *J* = 5.8 Hz), 2.53 (4H, t, *J* = 5.4 Hz), 1.77-1.54 (m, 4H), 1.44-1.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 23.2, 26.4, 54.3, 56.8, 68.6, 116.8, 121.7, 124.2, 124.4, 127.2, 127.6, 128.1, 130.4, 133.2, 133.6, 140.9, 146.4, 148.2, 152.6, 151.2, 188.4; ES-MS (*m*/*z*): 409 $(M+H)^+$; Anal. calcd. (found) % for $C_{22}H_{21}N_4O_2Cl$: C, 64.62 (64.58); H, 5.18 (5.14); N, 13.70 (13.73).

(*E*)-8-Chloro-6-(2-(pyrrolidin-1-yl)ethoxyimino)-indolo[2,1-*b*]quinazolin-12(6*H*)-one (5g): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$) 8.52 (1 H, d, *J* = 8.1 Hz), 8.48 (1H, d, *J* = 7.4 Hz), 8.09 (1H, d, *J* = 7.3 Hz), 7.96-7.84 (2H, m), 7.81-7.75 (2H, m), 4.83 (2H, t, *J* = 5.6 Hz), 3.15 (2H, t, *J* = 5.4 Hz), 2.71-2.75 (4 H, m), 1.86-1.70 (4 H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 25.3, 55.6, 58.2, 68.4, 116.2, 121.6, 124.2, 124.3, 127.2, 127.4, 128.1, 130.3, 133.2, 133.4, 140.9, 146.3, 148.2, 152.5, 151.2, 188.5; ES-MS (*m*/*z*): 395 $(M+H)^+$; Anal. calcd. (found) % for $C_{21}H_{19}N_4O_2Cl$: C, 63.88 (63.84); H, 4.85 (4.81); N, 14.19 (14.22).

(*E*)-8-Chloro-6-((3-chloropropoxy)methylene)indolo-[2,1-*b*]quinazolin-12(6*H*)-one (5h): White solid; m.p.: 179 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.63 (1 H, d, *J* = 8.3 Hz), 8.48 (1H, d, *J* = 7.4 Hz), 8.27 (1H, d, *J* = 7.6 Hz) 7.99 (1H, d, *J* = 8.0 Hz), 7.84-7.78 (1 H, m), 7.76-7.56 (2H, m), 4.81 (2H, t, *J* = 6.0 Hz), 3.75 (2H, t, *J* = 6.4 Hz), 2.41-2.38 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 29.4, 40.2, 64.6, 116.5, 121.8, 123.5, 124.9, 127.6, 127.9, 128.3, 130.0, 133.9, 134.4, 141.3, 146.5, 148.6, 152.7, 151.6, 188.4; ES-MS (*m*/*z*): 373 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{14}N_2O_2Cl_2$: C, 61.14 (61.10); H, 3.78 (3.75); N, 7.51 (7.55).

(*E*)-8-Chloro-6-(2-(diisopropylamino)ethoxyimino)-indolo[2,1-*b*]quinazolin-12(6*H*)-one (5i): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.63 (1 H, d, *J* = 7.8 Hz), 8.51 (1H, d, *J* = 7.9 Hz), 8.17 (1H, d, *J* = 7.6*Hz*), 7.90-7.77 (2H, m), 7.60-7.41 (2H, m), 4.61 (2H, t, *J* = 6.7 Hz), 3.13-3.67 (2H, m), 2.96 (2H, t, *J* = 6.6 Hz), 1.07 (s, 1H), 1.05 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.4, 40.2, 50.3, 64.4, 116.8, 122.1, 124.0, 124.5, 127.2, 127.8, 128.1, 130.2, 133.6, 134.1, 141.2, 146.4, 148.6, 152.4, 151.2, 188.2; ES-MS (*m*/*z*): 425 $(M+H)^+$; Anal. calcd. (found) % for $C_{23}H_{25}N_4O_2Cl$: C, 65.01 (65.00); H, 5.93 (5.89); N, 13.19 (13.22).

(*E*)-8-Chloro-6-(2-(dimethylamino)ethoxyimino)-indolo[2,1-*b*]quinazolin-12(6*H*)-one (5j): viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.68 (1 H, d, *J* = 8.3 Hz), 8.54 (1H, d, *J* = 8.0 Hz), 8.21 (1H, d, *J* = 7.6 Hz), 7.90-7.77 (2H, m), 7.81-7.76 (2H, m), 4.74 (2H, t, *J* = 5.8 Hz), 2.84 (2H, t, *J* = 5.7 Hz), 2.40 (6*H*, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 44.2,

58.4, 66.6, 116.6, 121.8, 124.1, 124.3, 127.0, 127.8, 128.1, 130.4, 133.2, 133.8, 140.9, 146.8, 148.6, 152.4, 151.2, 188.6; ES-MS (*m/z*): 369 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{17}N_4O_2Cl$: C, 61.87 (61.84); H, 4.65 (4.61); N, 15.19 (15.22).

(*E*)-8-Nitro-6-(2-(piperidin-1-yl)ethoxyimino)indolo-[2,1-*b*]quinazolin-12(6*H*)-one (5k): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.64-8.58 (2H, m), 8.54 (1H, s), 8.38 (1H, d, *J* = 7.3 Hz), 7.82 (1H, d, *J* = 7.1 Hz), 7.74-7.69 (1 H, m), 7.64-7.52 (2H, m), 7.38 (1 H, t, *J* = 7.58 Hz), 4.74 (2H, t, *J* = 7.6 Hz), 2.86 (2H, t, *J* = 5.8 Hz), 2.55-2.48 (4H, m), 1.75-1.56 (m, 4H), 1.46-1.44 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 24.1, 25.8, 54.5, 56.9, 68.4, 116.5, 121.2, 120.8, 124.1, 126.4, 132.3, 133.4, 146.4, 147.1, 154.3, 160.6, 186.4; ES-MS (*m/z*): 420 $(M+H)^+$; Anal. calcd. for $C_{22}H_{21}N_5O_4Cl$: C, 63.00 (62.96); H, 5.05 (5.01); N, 16.70 (16.73).

(*E*)-8-Nitro-6-(2-(pyrrolidin-1-yl)ethoxyimino)indolo-[2,1-*b*]quinazolin-12(6*H*)-one (5l): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.29-8.23 (2H, m), 8.10 (1H, s), 7.81 (1H, d, *J* = 7.5 Hz), 7.53-7.41 (2H, m), 7.34-7.21 (1H, m), 4.84 (2H, t, *J* = 5.7 Hz), 3.12 (2H, t, *J* = 5.7 Hz), 2.71-2.75 (4H, m), 1.88-1.71 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 24.7, 55.2, 56.2, 68.2, 115.8, 121.3, 120.8, 124.1, 126.3, 132.3, 133.4, 146.5, 147.1, 154.1, 160.6, 186.2; ES-MS (*m/z*): 406 $(M+H)^+$; Anal. calcd. (found) % for $C_{21}H_{19}N_5O_4$: C, 62.22 (62.18); H, 4.72 (4.69); N, 17.27 (17.30).

(*E*)-6-(2-(Dimethylamino)ethoxyimino)-8-nitroindolo-[2,1-*b*]quinazolin-12(6*H*)-one (5n): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.35-8.29 (2H, m), 8.18 (1H, s), 8.02 (1H, d, *J* = 7.4 Hz), 7.63-7.51 (2H, m), 7.44-7.36 (2H, m), 4.75 (2H, t, *J* = 5.8 Hz), 2.85 (2H, t, *J* = 5.7 Hz), 2.41 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 43.6, 58.2, 68.6, 116.1, 120.5, 121.8, 124.4, 126.4, 132.2, 133.8, 146.4, 147.2, 154.3, 160.4, 186.1; ESMS (*m/z*): 380 $(M+H)^+$; Anal. calcd. (found) % for $C_{19}H_{17}N_5O_4$: C, 60.15 (60.11); H, 4.52 (4.47); N, 18.46 (18.49).

(*E*)-6-(2-(Diisopropylamino)ethoxyimino)-8-nitro-indolo[2,1-*b*]quinazolin-12(6*H*)-one (5o): Viscous oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.56-8.47 (2H, m), 8.24 (1H, s), 7.81-7.74 (2H, m), 7.60-7.48 (2H, m), 4.61 (2H, t, *J* = 6.7 Hz), 3.13-3.67 (2H, m), 2.96 (2H, t, *J* = 6.6 Hz), 1.07 (s, 1H), 1.05 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.8, 41.6, 51.8, 64.6, 116.8, 120.2, 121.6, 124.4, 127.4, 132.4, 133.8, 145.4, 146.2, 154.3, 161.4, 186.3; ES-MS (*m/z*): 436 $(M+H)^+$; Anal. calcd. (found) % for $C_{23}H_{25}N_5O_4$: C, 63.44 (63.40); H, 5.79 (5.74); N, 16.08 (16.11).

RESULTS AND DISCUSSION

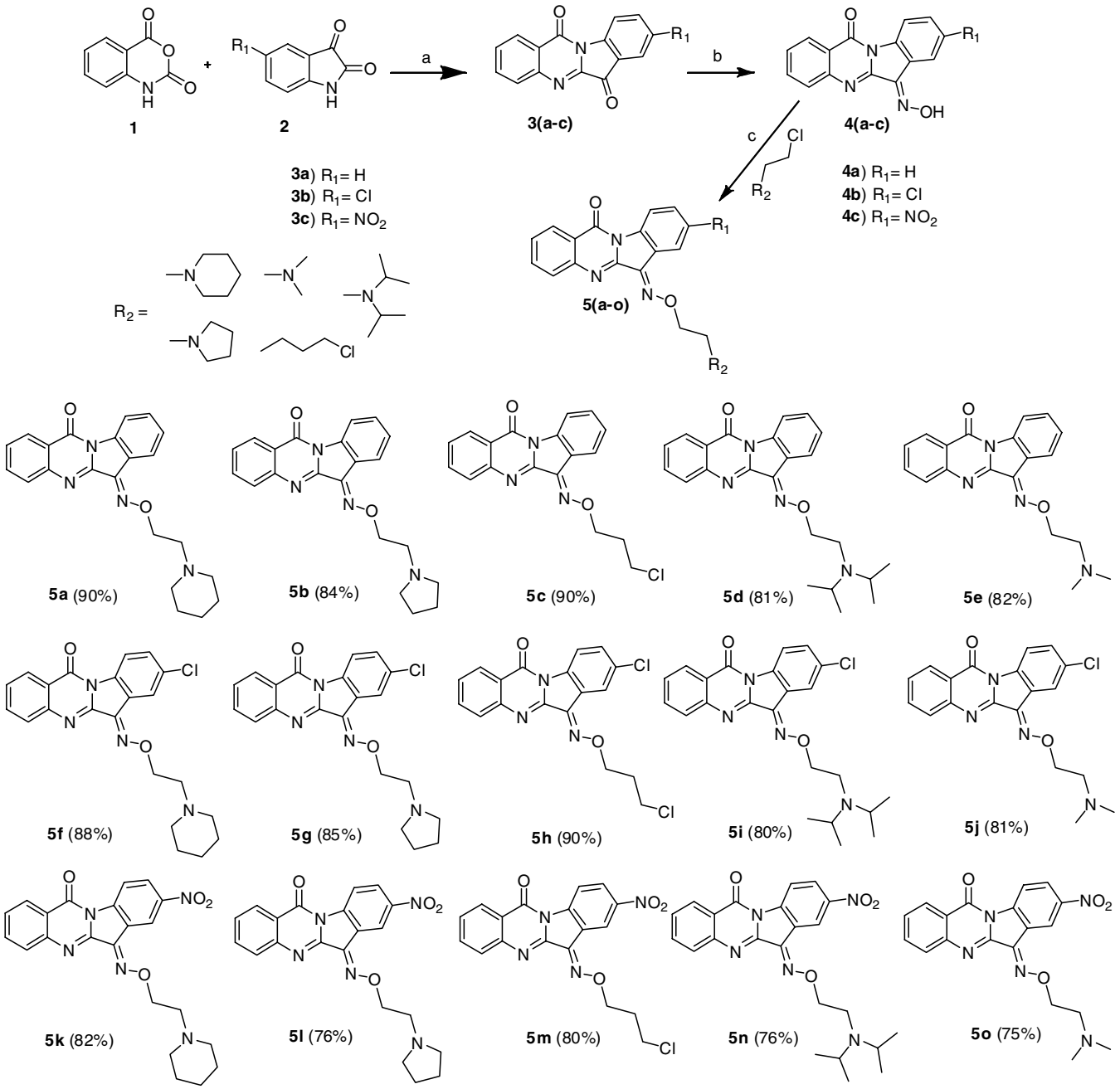
Earlier, we reported the first green synthesis of tryptanthrin derivatives from isatoic anhydride and isatins [31]. The starting point of our work was to prepare three substituted tryptanthrin analogues (**3a-c**). Tryptanthrins (**3a-c**) were synthesized by our earlier reported procedure, using commercially available substituted isatins and isatoic anhydride in water [31]. β-Cyclodextrin was employed as catalyst in aqueous medium. Tryptanthrins (**3a-c**) were isolated in excellent yields (up to 90%) and negligible chromatographic purification was required at this stage. Then we focus on conver-sion of ketonic group of tryptanthrin into oxime functionality and achieved this *via* reaction of tryptanthrins with hydroxylamine hydrochloride using KOH pallets as base in **Scheme-I**.

Corresponding oxime derivatives were isolated in excellent yield (**4a-c** upto 92%). Formation of oxime analogue was confirmed by IR, ^{1}H & ^{13}C NMR spectroscopy. Tryptanthrin oxime derivatives were precipitated in reaction medium and separated by filteration and purified by washing with water and ethanol. No chromatographic purification was required at this stage.

Next strategy was to attach pharmacophoric aminoalkyl side chains in selected natural product scaffold. To achieve this hydroxyl group of oxime derivative was alkylated with various aminoalkyl chains using a strong base as NaH in DMF. Thin layer chromatography in silica was used to check the progress of reaction. Surprisingly, it was observed that derivative with nitro substitution on tryptanthrin nucleus having poor solubility and probably due to this reason the isolated yields of products (**5k-l**) were low. The alkylated tryptanthrin derivatives were isolated as oily products. Compounds (**5a-o**) were formed in good to excellent yields and all the synthesized aminoalkyl derivatives were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy, mass spectroscopy and elemental analysis. These aminoalkyl derivatives were further evaluated for their pharmacological activity.

Biological activity: All the synthesized compounds (**3a-c, 4a-c, 5a-o**) were screened *in vitro* for their antimalarial potential against *P. falciparum* 3D7 and *P. falciparum* k1 which is a chloroquine resistant cell line for *in vitro* efficacy. Most of synthesized aminoalkyl derivatives (**5c-p**) have shown superior *in vitro* antimalarial activity. Table-1 presents the *in vitro* antimalarial assay results of the intermediates starting tryptanthrins (**3a-c**), oxime analogues (**4a-c**) and the target compounds (**5a-c**) as well as the control chloroquin. Inhibitory potential is represented in form IC_{50} values. The cytotoxic evaluation of these compounds was carried out on VERO cell line, the results are shown in Table-1. On the basis of antimalarial activity and cytotoxic activities, the calculated selectivity indices (SI values) (CC_{50}/IC_{50}) have shown that all the compounds active against malaria have high degree of safety. Compound **5f** has shown the best selectivity index (5490) as well as potent antimalarial activity. Among all the synthesized antimalarial compounds, 12 compounds have shown median cytotoxic concentration > 500 µg/mL (Table-1).

An observation of activity data reveals that the aminoalkyl derivatives of tryptanthrin with cyclic amines showed better antimalarial activity than parent natural products (**3a-c**) as well as corresponding oxime (**4a-c**) derivatives. Parent natural product (tryptanthrins) **3a, 3b** and **3c** were showing IC_{50} values of 160, 150 and 80 nm, respectively against 3D7 strain. A comparative examination showed that tryptanthrin with nitro substitution (**3c**) was the most active tryptanthrin derivative. Intere-stingly, these molecules have also exhibited very good inhibitory property against resistance *P. falciperum* K1 strain with IC_{50} value of 140, 120 and 30 nm, respectively. Notably the most active nitro derivative **3c** was found to more toxic with compared to other analogues **3a** and **3b** with its CC_{50} value of 32.48 µg/mL and SI index of 3248.



Scheme-I: Synthesis of tryptanthrin derivatives. **Reagent and condition:** (a) β-cyclodextrin, water, stirring, room temperature; (b) toluene, hydroxylamine hydrochloride, refluxing, 12 h; (c) dry acetone, K_2CO_3, aminoalkyl chain, refluxing

The target compounds 15 aminoalkyl derivatives (**5a-o**) have showed excellent antimalarial activity against both 3D7 strain as well as resistant k1 species. It is observed that compounds **5a, 5f** and **5k** with piperidine ring attached with tryptanthrin exhibited excellent activity against both the strains with the IC_{50} value as low as 10 nm in nitro substituted (**5k**) derivative in both the strains. Target compounds **5b, 5g** and **5l** with pyrroliodine ring have also shown excellent antimalarial potential. Noticeably these compounds were also killing resistant K1 strain as well with IC_{50} value of 30 nm. Compounds **5e, 5j** and **5o** having *N*,*N*-dimethyl substitution were showing IC_{50} value of 130, 120 and 70 nm, respectively against resistant strain. The important point at this stage was that most active compounds (**5k, 5i** and **5o**) were very safe with selectivity index value of more than 500. It is found that the molecules with chloro propyl chain (**5c, 5h** and **5m**) were less active against both the strains, with higher values IC_{50}. The only consistent conclusion in this regard is that none of the compounds with isopropyl aminoalkyl chain have significantly low activity.

Tryptanthrin oximes **4a-c** showed *in vitro* antimalarial activity comparable to tryptanthrins (**3a-c**), against both the strains. The most active oxime derivative was **4a**, with IC_{50} value of 80 and 30 nm against *P. falciparum* 3D7 and K1 strain, respectively. The oxime intermediate **4b** (chloro substitution) showed moderate antimalarial activity, comparable to that of the oximes **4a** and **4c**. This would suggest that for these heterocyclic scaffolds, the Cl group at 8-position does not exhibit potential activity instead that nitro group is more potent for

TABLE-1
ANTIMALARIAL ACTIVITY OF
SYNTHESIZED AMINOALKYL DERIVATIVES

Compd.	IC_{50} (nM) 3D7	IC_{50} (nM) p.f. k1	CC_{50}	SI
5a	100	1300	44.99	31.03
5b	150	30	> 500	> 3333.33
5c	60	30	31.48	524.67
5d	3660	9870	133.47	36.47
5e	140	130	> 500	> 3571.43
5f	70	100	384.31	5490.14
5g	110	100	> 500	> 4545.46
5h	11190	18570	307.34	27.47
5i	2988	3210	> 500	167.79
5j	2785	120	> 500	–
5k	10	30	> 500	> 6250
5l	30	30	34.37	1145.67
5m	10	ND	10.62	1062
5n	150	120	> 500	> 3333.33
5o	1450	70	> 500	> 5000
3a	160	120	> 500	> 3125
3b	150	140	> 500	> 3333.33
3c	80	120	32.48	3248
4a	80	30	> 500	> 3333.33
4b	15540	1390	> 500	> 196.85
4c	120	80	> 500	> 4166.67

antimalarial activity. However, it was apparent that this significant improvement in activity was not universal, as shown by the fact that the compounds with *N*,*N*-dimethyl and *N*,*N*-diisopropyl substituions (compounds **5e, 5j, 5o, 5d, 5i** and **5n**) does not have similar level of *in vitro* antimalarial activities. Also notable was that intermediate **3a** and **3b** having parent tryptanthrin natural product nucleus also showed some moderate activity in comparison to their aminoalkyl counterpart (**5a** and **5b**) with IC_{50} values of **150** nm and **160** nm against 3D7 *P. falciparum*.

Conclusion

The present work demonstrates a natural product inspired multistep synthesis of a series of aminoalkyl chain substituted analogues of natural product tryptanthrin in excellent yields. Synthesized compounds were evaluated against *P. falciparum* in both sensitive 3D7 and resistant k1 strain. Most of the compounds among compounds synthesized showed significant antimalarial property at with their IC_{50} value in the low nanomolar range. The synthesized target compounds were also found to be safe with high value of SI index. Results showed that derivatives with nitro substitution posses better antimalarial activity. Our synthesized natural product derived analogues were found to be more potent antimalarial agents than the parent natural product scaffold. The cytotoxicity as well as selectivity of synthesized compounds shows that synthesized compounds are more selective and very safe. Further *in vivo* exploration of this study is currently underway.

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